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Dietary Nitrogen and Energy Metabolism in the Ruminant: Effect of Source of Nitrogen.

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DIETARY NITROGEN AND ENERGY METABOLISM IN THE RUMINANT: EFFECT OF SOURCE OF NITROGEN

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List of Abbreviations

A.I. ..... artificial insemination

ADG ..... average daily gain

BCS ..... body condition score

BOHB ..... β-hydroxybutyrate

BW ..... body weight

CP ..... crude protein

CV .....coefficient of variation

DIP ..... rumen degradable intake protein

DM ..... dry matter

DMI .....dry matter intake

FCM ..... fat corrected milk

N ..... nitrogen

NEFA ..... nonesterified fatty acid

NPN ..... nonprotein nitrogen

OULT ..... oral urea load test

PMR ..... partial mixed ration

SBM ..... soybean meal

TMR ..... total mixed ration

T_3 ..... triiodothyronine

T_4 ..... thyroxine

UIP ..... rumen undegradable intake protein

Abstract

Three experiments were conducted to determine the effects of dietary nitrogen on the energy status of ruminants and the feasibility of using L-carnitine to prevent the negative effects associated with overfeeding nitrogen. The objectives of the first experiment were to monitor the nitrogen and energy status of cows grazing ryegrass pastures through the measurement of key blood metabolites and insulin and to determine the protein supplementation scheme which would maximize milk yield and maintain plasma urea nitrogen levels below dangerous levels. In the first experiment, the protein supplement that supplied excess total protein exerted negative effects on body condition and plasma nitrogen metabolites but had a minimal effect on plasma energy metabolites and insulin. The nutritional status of the cows post-partum and prior to grazing seemed to have a greater effect compared with the dietary protein supplements on the ability of the cow to maintain condition during early lactation. The second experiment was conducted to investigate the effects of intravenous administration of L-carnitine in sheep, and to determine its ability to ameliorate subclinical hyperammonemia experimentally-induced using an oral urea load test. Results indicated that intravenous administration of L-carnitine (12.72 mmol/kg$^{.75}$ BW) increased plasma L-carnitine, glucose and nonesterified fatty acid concentrations in sheep. Additionally, when L-carnitine (6.36 mmol/kg$^{.75}$ BW) was administered intravenously 30 min preceding an oral urea load test, the development of hyperammonemia was prevented. The last experiment was conducted to determine the influence of supplemental L-carnitine on growth and metabolic criteria of growing lambs fed a diet high in nonprotein nitrogen (compromising 50% of total dietary nitrogen).

Supplementation of L-carnitine increased plasma total L-carnitine concentrations and lowered plasma ammonia nitrogen concentrations during an oral urea load test but had minimal effects on ruminal and blood characteristics. Nonprotein nitrogen negatively affected both production and metabolic parameters and exerted a greater influence on the energy status of sheep compared with L-carnitine. Although L-carnitine had minimal effects, the prevention of hyperammonemia during two of the oral urea load tests suggests that further research is warranted into the feasibility of using L-carnitine to ameliorate hyperammonemia.

Chapter 1

Introduction

Interrelationships exist between the various metabolic systems in the body (Murray et al., 1990). As a result, the variable aspects of metabolism are affected when physiological changes occur such as pregnancy, lactation, and starvation or when nutritional changes occur such as nutritional deficiencies or excesses (Murray et al., 1990). The metabolism of protein and energy are two factors in intermediary metabolism that are linked to each other. Both are necessary for normal growth and maintenance; however, changes in one could affect the other. Studies have shown that when substantial amounts of nonprotein nitrogen or highly-soluble nitrogen are fed to ruminants, the production performance is suboptimal (Chalupa, 1972; Barej et al., 1979; McCormick et al., 1994). Researchers have proposed that the resulting suboptimal performance may be attributed to elevated levels of plasma ammonia which results in a subclinical ammonia toxicity, that while not fatal, causes derangements in intermediary metabolism (Chalupa, 1972; Spires and Clark, 1979; Visek, 1984). It is possible that energy metabolism is affected by the development of subclinical ammonia toxicity resulting in an increase in glycogenolysis and a decrease in glucose utilization by insulin-sensitive tissues (Spires and Clark, 1979; Fernandez et al., 1988).

Because of the diversity of production practices, it is necessary to characterize the relationship between dietary nitrogen sources and energy during different production schemes such as feeding ruminants a nonprotein nitrogen source, broiler litter, or grazing pastures that are high in soluble nitrogen (i.e., ryegrass). Additionally, the naturally-

occurring, vitamin-like compound, L-carnitine has been shown to reduce experimentally-induced ammonia-related deaths in mice (Grisolia et al., 1984; O'Connor et al., 1987) and to increase the tolerance of Channel catfish to environmental ammonia (Burtle and Newton, 1991). L-carnitine may have the potential to prevent ammonia-related deaths in ruminants. Three experiments were conducted to determine the effects of excess dietary nitrogen on the energy status of ruminants, and the feasibility of using L-carnitine to prevent the negative effects associated with overfeeding nitrogen. The first experiment was conducted to monitor the metabolic status of cows grazing ryegrass pastures through the measurement of key blood metabolites and determined the effect of dietary nitrogen source and concentration in grain supplements that will maximize milk yield and maintain plasma urea nitrogen levels below dangerous levels (< 20 mg/dL) for cows on all ryegrass forage. The second experiment investigated the effects of intravenous administration of L-carnitine in sheep, and determined its ability to ameliorate experimentally-induced subclinical hyperammonemia. The final experiment was conducted to determine the effects of supplemental L-carnitine in growing lambs fed a diet high in nonprotein nitrogen.

Chapter 2

Review of Literature

Suboptimal performance due to excess nitrogen

Introduction. Ruminants have the unique ability to survive and even be productive without a source of preformed dietary protein (Church, 1988). When a source of nitrogen (N) is provided in the diet, the microorganisms in the rumen are able to produce microbial protein during rumen fermentation (NRC, 1988). As a result of the microorganisms being washed out of the rumen and then digested and absorbed in the small intestine, the ruminant is supplied with the amino acids necessary for growth and production; thus, a synergistic relationship exists between the ruminant and rumen microbial population (Church, 1988; NRC, 1988). As a result of this synergistic relationship, nitrogen can be supplied to the rumen by either dietary crude protein (CP), nonprotein nitrogen (NPN), or a combination of both. Dietary CP is composed of two fractions: rumen undegradable intake protein (UIP) and rumen degradable intake protein (DIP) (NRC, 1988). Rumen undegradable intake protein, also known as "escape" or "bypass" protein, escapes ruminal degradation to an extent and passes to the omasum and abomasum (NRC, 1984). In contrast, rumen degradable intake protein is degraded in the rumen to ammonia to a greater extent compared with UIP (NRC, 1988). Nonprotein nitrogen compounds by definition are not proteins but are nitrogen-containing compounds (Maynard et al., 1979). Compounds categorized in this group include purines, pyrimidines, amino acids, amides, and nitrates (Maynard, 1979). Of this group, amides and amino acids are of nutritional

importance because they constitute nearly one third of the total nitrogen in pasture and early-cut hay and approximately 50% of the nitrogen in silage crops (Maynard et al., 1979).

Other compounds classified as NPN include the feed additives urea, biuret, uric acid, and ammoniated products (Maynard et al., 1979). Urea is the most commonly used NPN feed additive used in ruminant diets (Church, 1988). Urea can replace a portion of the CP in the diet, and thereby lowering the cost of protein supplementation (NRC, 1984; Church, 1988). Soluble protein from immature pastures and NPN (i.e., urea) are both highly degraded in the rumen and rapidly converted to ammonia, which in excess, can result in ammonia toxicity (Maynard et al., 1979; Haliburton and Morgan, 1989). Ammonia toxicity, either acute or subacute, causes derangements in intermediary metabolism (Chalupa, 1972; Spires and Clark, 1979; Visek, 1984; Haliburton and Morgan, 1989) which, eventually, could negatively affect the performance of production livestock (NRC, 1976; Visek, 1984).

Production. Undernutrition has been shown to have detrimental affects on livestock production (Robinson, 1990). However, inconsistent results have been observed with the overfeeding of protein in production animals. Some studies conducted with dairy cows (Fenderson and Bergen, 1976; Polan et al., 1976; Kertz et al., 1982) and goats (Ciszuk and Linderber, 1988; McGregor and Hodge, 1988) have shown a decrease in feed intake due to high levels of CP, whereas, other studies in dairy cows (Claypool et al., 1980; Oldham and Smith, 1982; Blauwiekel and Kincaid, 1986) and goats (Singhal and Mudgal, 1984; Lu et al., 1990; Sahlu et al., 1992b, 1993; Fernandez et al., 1997) have

shown no differences or even increases in feed intake. Kertz et al. (1982) suggested that the depression in feed intake associated with excess nitrogen could be due to either rejection of a ration because of the "newness" of added urea into the diet or limitation of intake through a negative feedback mechanism due to the effects of subclinical ammonia toxicity. Claypool et al. (1980) observed higher feed intakes in early lactation dairy cows fed 16.3 and 19.3% CP on a DM basis. However, in this experiment, soybean meal instead of urea was used to increase the CP content of the rations which, the authors suggest, resulted in a higher palatability of the ration and therefore a higher intake (Claypool et al., 1980).

A large excess of dietary protein may decrease the energy supply because excess protein must be deaminated to ammonia and converted to urea for excretion (NRC, 1988). Blaxter (1962) calculated that each gram of nitrogen excreted requires 5.88 kcal and that each gram of nitrogen excreted as urea in urine represents a theoretical excretion of 5.41 kcal. Oldham (1984) found that energy lost in the synthesis of urea from CP and the excretion of urea reduced milk output. Responses in milk production to excess dietary protein, as with feed intake, are variable. Several studies using dairy goats found no changes in milk yield due to supplementation of NPN into the rations of lactating does (Singhal and Mudgal, 1984; Ciszuk and Lindberg, 1988; Lu et al., 1990; Fernandez et al., 1997). McGregor and Hodge (1988) reported a 39% decrease in milk production in early-lactating Australian feral does fed an oat-based diet supplemented with urea. In contrast, milk yields tended to increase in dairy cows fed rations containing up to 19% CP, (Sparrow et al., 1973; Chandler et al., 1976; Roffler et al., 1976; Cressman et al., 1977;

Blauwiekel and Kincaid, 1986). Typically, milk production increases in response to increased protein intake (NRC, 1988). In relation to milk production, Hermansen et al. (1994) investigated the effects of nitrogen fertilizer on the properties of milk. Danish Holsteins grazing fertilized (240 kg N/ha) white clover and ryegrass pasture showed a decrease in total milk protein concentration and no effect on the renneting properties of milk (important for cheese production) when compared to Holsteins grazing unfertilized pastures (Hermansen et al., 1994).

In addition to milk yield, mohair production is also influenced by the amount of CP in the diet (Sahlu et al., 1992). Angora goats fed diets containing 19% CP had greater grease and clean mohair weights; however, mohair fiber diameter was not affected by the level of CP (Sahlu et al., 1992). Similar results were seen in a similar study conducted by Sahlu et al. (1993) when Angora goats were fed diets containing 15 and 20% CP.

Reproduction. Supplementation of dietary protein during early pregnancy and/or prior to mating may have a detrimental effect on reproductive performance (Zavy and Geisert, 1994). Cattle consuming excess rumen degradable protein either through grain supplements or grazing immature pastures experienced decreased number of first service conception rates, (Jordan and Swanson, 1979; Wilson et al., 1985; Randel, 1990; Elrod and Butler, 1991) increased embryo mortality rates (Zavy and Geisert, 1994), and increased number of days open (Sonderegger and Schurch, 1977). In contrast, in a study using 146 cows fed either 15 or 20% CP, Howard et al. (1987) reported no differences in services per conception or days open. These results agree with several studies in dairy cows which investigated feeding urea at levels which provided up to one-half of the crude

protein (NRC, 1976). These studies, which compiled close to 100,00 observations, were conducted to determine the relationship of urea to milk production and reproduction (NRC, 1976). These studies concluded that feeding urea at levels to provide up to one-half of the crude protein had no negative effects on milk production or reproduction (NRC, 1976). Blanchard et al. (1990) suggested that the infertility in cows fed excess nitrogen could be due to fertilization failure or early degeneration of embryos. In support of this, Elrod and Butler (1991) determined that excess degradable protein lowers uterine pH and therefore depresses fertility.

Two other mechanisms have also been proposed as the link between excess protein and infertility. The first is that excessive CP leads to increased ruminal and blood ammonia and urea nitrogen concentrations resulting in a local toxic effect on sperm, ovum, or developing embryo (Jordan and Swanson, 1979a; Jordan et al., 1983; Chalupa, 1984). The second is that high urea nitrogen or ammonia concentrations may reduce luteinizing hormone binding to ovarian receptors, leading to a decrease in serum progesterone concentrations (Jordan et al., 1983). Folman et al. (1981) also postulated that reduced fertility may be contributing to lowered plasma progesterone. Another possible explanation for decreased fertility due to overfeeding protein may be an interaction between protein feeding and energy balance and not the absolute concentration of CP (Sonderegger and Schurch, 1977). Although milk production increases with increases in protein intake, the milk produced can be at the expense of body reserves particularly in early-lactation cattle which are already in negative energy balance (Oldham, 1984). Wiltbank (1970) showed that cows which had body fat reserves at parturition but

which lost weight when inadequately supplied with energy had the poorest rate of conception at first service. Other studies indirectly support an interaction between protein and energy. These studies showed that cows fed excess protein exhibited no negative reproductive response, however, the cows lost little body condition (Carroll et al., 1994), were nonlactating (Garcia-Bojalil et al., 1994), or were producing milk at a moderately low level (Howard et al., 1987). A study in mice also indicted that the absolute concentration of CP was not as important in reproduction as was the CP to crude fat ratio (Knapka et al., 1977). A study using mice at the National Institutes of Health showed that neither protein nor fat alone had significant effects on number of litters and pups born, number of litters and pups weaned, weanling mortality, or weanling weight; however, the interaction of fat and protein was significant (Knapka et al., 1977).

Hyperammonemia

Introduction. When large amounts of NPN are added to ruminant diets, the rumen microbial population is unable to incorporate all of the ammonia (NH_3) produced into microbial protein (Visek, 1984). The ammonia is then absorbed across the rumen epithelium into the portal and lymphatic circulation and can result in hyperammonemia (Chalmers et al., 1971; Chalupa, 1972; Bartley et al., 1976, 1981; Spires and Clark, 1979; Symonds et al., 1981; Owens and Bergen, 1983; Casteel and Cook, 1984; Visek, 1984; Huntington, 1986, 1990; Huntington and Eiseman, 1988; Haliburton and Morgan, 1989; Fernandez et al., 1990). Hyperammonemia, also known as ammonia toxicity, is a poorly understood metabolic disease which is known to cause derangements in intermediary metabolism (Chalupa, 1972; Spires and Clark, 1979; Visek, 1984). Specifically,

ruminants experience hyperglycemia (Bartley et al., 1976; Emmanuel et al., 1982; Fernandez et al., 1988, 1990a,b) because of increased glycogenolysis (Spires and Clark, 1979; Fernandez et al., 1990a) and reduced glucose uptake resulting from insulin insufficiency (Sener et al., 1978; Fernandez et al., 1988, 1990a,b). In addition, other circulating metabolites such as lactate (Garwacki et al., 1979; Emmanuel and Edjtehadi, 1981; Fernandez et al., 1988, 1990, 1991), urea (Bartley et al., 1976, 1981; Spires and Clark, 1979; Orzechowski et al., 1988; Fernandez et al., 1989, 1990), and NEFAs (Grisolia et al., 1984; Fernandez et al., 1988, 1990) increase during hyperammonemia. Subclinical or subacute ammonia toxicity, although not fatal, may result in the suboptimal performance of production livestock (Chalupa, 1972; Visek, 1984; Fernandez et al., 1988, 1997).

Hyperammonemia can be characterized by elevated levels of circulating ammonia (Visek, 1968, 1984; Chalupa, 1972; Casteel and Cook, 1984). These elevated levels of ammonia in circulation are associated with the rapid absorption of the nonionized form of ammonia (NH_3 vs NH_4^+) from the rumen under conditions of elevated pH and excess ammonia (Bartley et al., 1981; Huntington, 1986; Haliburton and Morgan, 1989). Ammonia intoxication produces clinical signs which can make diagnosis difficult (Visek, 1984). The use of blood ammonia cannot specify the severity of the intoxication because the correlation of the degree of hyperammonemia with blood ammonia concentrations is poor (Visek, 1984). Currently, ammonia toxicity is untreatable and is almost 100% lethal in acute cases (Casteel and Cook, 1984; Visek, 1984; Haliburton and Morgan, 1989). The only effective method of treating acute cases if hyperammonemia is total rumen evacuation

(Bartley et al., 1976, 1981; Casteel and Cook, 1984; Haliburton and Morgan, 1989); however, in a production setting, this method is not practical.

Ammonia effects on tissues. Ammonia is a weak electrolyte which exists in two forms in equilibrium: the ionic form (NH_4^+) and the nonionic form (NH_3) (Visek, 1984). Ammonia is both a building block for purines, pyrimidines, nonessential amino acids, and is also a waste product known for its toxicity to cells (Visek, 1984; Murray et al., 1990). The body disposes of ammonia by converting it to the nontoxic compound urea in the liver via the urea cycle (Murray et al., 1990). Ammonia in the body arises from several sources: production from glutamine by the kidney, production by endogenous and bacterial enzymes within the alimentary tract, and in ruminants, absorption from the rumen (Visek, 1972, 1984; Murray et al., 1990). It is only when the liver, which performs the detoxification of ammonia, is overwhelmed that cells and tissues experience the toxic effects of ammonia (Visek, 1968; Symonds et al., 1981).

Although it is known that ammonia is toxic to cells, the precise mechanism by which ammonia exerts its effects is poorly understood (Chalupa, 1972; Spires and Clark, 1979; Visek, 1984). Exposure to ammonia shortens the lifespan of cells which then must be replaced with greater frequency and as a result, the rate of metabolic and biosynthetic processes must be maintained at a higher level (Dang and Visek, 1968). Dang and Visek (1968) found that in rabbits, urease injections caused a decrease in white cell count, hematocrit, and hemoglobin as well as an increase in blood ammonia. Not found exclusively in the portal blood, ammonia can diffuse from the rumen directly into the lymphatic system and carotid blood, thus bypassing the liver in both cases (Bartley et al.,

1981). In contrast to the liver, the brain removes ammonia by the formation of glutamine (Murray et al., 1990). Lin and Raabe (1985) showed that in the cat, systemic ammonia intoxication produced metabolic changes in the spinal cord similar to those in the cerebral cortex, specifically increased levels of NH_4^+, glutamine, glutamine/glutamate ratio, lactate, and the lactate/pyruvate ratio. Hyperammonemia causes seizures in mice and alters the levels of brain energy metabolites by decreasing phosphocreatine and ATP while increasing ADP, AMP, and lactate (Igisu et al., 1995). Severe ammonia toxicity can quickly result in a coma and death (Visek, 1984).

Ammonia effects on metabolism. Since ammonia toxicity is a metabolic disorder, ammonia's effects on metabolism are diverse. Hyperglycemia, which is a common occurence during hyperammonemia in both animals and humans, may be attributed to a decrease in glucose utilization by insulin-sensitive tissues (Sener et al., 1978; Barej and Harmeyer, 1979; Emmanuel and Edjtehadi, 1981; Emmanuel et al., 1982; Fernandez et al., 1988). In vitro studies using pancreatic cells from golden hamsters and albino rats have shown that when ammonia (NH_4^+) was introduced, insulin secretion was inhibited (Feldman and Lebovitz; 1971; Sener et al., 1978). Studies using sheep (Barej and Harmeyer, 1979; Emmanuel et al., 1982), rats (Fausel and Mulloy, 1983), steers (Fernandez et al., 1988), and dogs (Strombeck et al., 1978) have found that administration of ammonia also decreased circulating insulin concentrations. Moreover, Fernandez et al. (1988) reported that an infusion of ammonium chloride decreased plasma insulin concentrations by 26 to 46%, however, when the infusion ceased, the plasma

insulin levels quickly increased 89 to 122%. Uremic patients also show a resistance to insulin (Defronzo et al., 1973; Feldman and Singer, 1975).

In addition to insulin, glucagon, another hormone involved in carbohydrate metabolism, has been variable in its response to ammonia. In steers, plasma glucagon concentrations decreased during ammonium chloride infusion (Fernandez et al., 1990). However, in dogs, ammonia solutions increased the production of glucagon (Strombeck et al., 1978). Catecholamines are also affected by ammonia toxicity. Using dogs, Strombeck et al. (1981) reported a 20-fold increase in the urinary excretion of catecholamines when the dogs were perfused with ammonia. Ammonia-urea toxicosis in sheep resulted in an increase in plasma epinephrine (Emmanuel et al., 1982). However, intravenous infusion of ammonium chloride in steers did not result in an increase in epinephrine, norepinephrine, or dopamine (Fernandez et al., 1988). Garwacki et al. (1979) suggested that not all the effects due to ammonia are mediated by epinephrine. The administration of propanolol, a β-receptor blocker, prevented the rise of blood pyruvate and lactate that were associated with the administration of epinephrine; however, this rise was still observed after the infusion of ammonium chloride (Garwacki et al., 1979).

Hyperammonemia also affects other blood metabolites. The urea cycle is a link between ammonia and urea concentrations in the plasma (Stryer, 1988). Ammonia is converted to urea via the reactions of the urea cycle, so increases in plasma urea are common during hyperammonemia (Visek, 1984). Plasma lactate concentrations were increased in sheep (Garwacki et al., 1979; Emmanuel and Edjtehadi, 1981) and steers (Symonds et al., 1981 ; Fernandez et al., 1988) by ammonia administration. Emmanuel

and Edjtehadi (1981) suggested that the accumulation of lactate was due to the impairment of oxidative phosphorylation and decarboxylation of pyruvate.

Lipid metabolism is also affected by ammonia toxicity. Studies in sheep (Garwacki et al., 1979) and steers (Fernandez et al., 1988, 1990) have shown that NEFA concentrations increase in response to ammonia. Garwacki et al. (1979) demonstrated that the increase in fatty acids was not totally mediated through epinephrine. The administration of phentolamine, an α-receptor blocker, significantly reduced the rise of NEFA which is associated with the administration of epinephrine; however, this rise was still observed after the infusion of ammonium chloride (Garwacki et al., 1979). In steers, NEFAs increased initially during the first hour of an ammonium chloride infusion, but then declined steadily (Fernandez et al., 1990). Fernandez et al. (1990) suggested that because the availability of glucose is reduced, the peripheral tissues are utilizing NEFAs to a greater extent.

The ruminal environment is also affected by excess ammonia. The rapid release of ammonia through hydrolysis can contribute to a rise in rumen pH (Church, 1988). Under conditions of rumen alkalosis, ammonia absorption is increased and contributes to high blood ammonia concentrations (Church, 1988).

Carnitine

Introduction. Carnitine (β-hydroxy-γ-trimethylaminobutyric acid) is a quaternary amine compound with a molecular weight of 161.2 daltons which is synthesized in the liver and kidney from the amino acids lysine and methionine (Machlin, 1991; Figure 2.1).

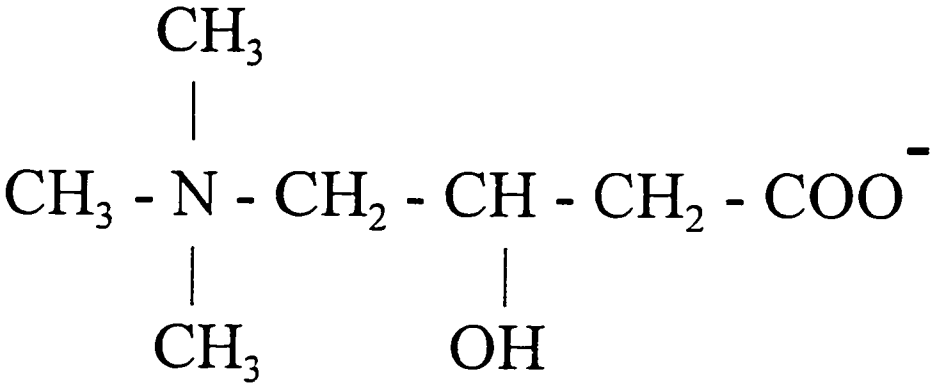

Figure 2.1. Structure of carnitine.

Other nutrients including iron, ascorbate, niacin, and vitamin B_6 are also required for L-carnitine synthesis (Machlin, 1991). Not all tissues can synthesize carnitine (i.e. cardiac and skeletal muscle) and must therefore depend on the transport of carnitine from other tissues (Machlin, 1991). Carnitine, in biological systems, exists in both the free form as well as carnitine esters (Owen et al., 1997). Although carnitine was first isolated by Gulewitsch and Kremberg in 1905, it wasn't until the 1950's that Fritz (1959) demonstrated the physiological and biochemical functions of carnitine. The well-established function of carnitine is its role in transporting long chain fatty acids from the cytosol into the matrix of the mitochondria where β-oxidation takes place (Stryer, 1988). Briefly, a long-chain acyl CoA molecule transfers the acyl group to the hydroxyl group of carnitine to form acyl carnitine with carnitine acyltransferase I being the catalyst. The acyl carnitine is then shuttled across the inner mitochondrial membrane by a translocase. Carnitine acyltransferase II then catalyzes the reaction to transfer the acyl group back to CoA on the matrix side of the membrane. The carnitine is then returned to the cytosolic side (Stryer, 1988). In addition to its role in fatty acid metabolism, evidence exists for other possible functions of carnitine. These roles include metabolism of branched chain amino acids (Bohles et al., 1984), thermogenesis in brown adipose tissue (Hahn and Skala, 1975), influence on the pyruvate dehydrogenase complex (Sugden and Holness, 1994), and shuttling the acyl groups of acyl-CoAs between tissue compartments (Bressler and Brendel, 1966).

Effects on production. Although carnitine is not considered to be an essential nutrient for mammals, studies in swine (Owen et al., 1996; Musser et al., 1997) and cattle

(Staples et al., 1975; LaCount et al., 1995; LaCount et al., 1996a,b) have shown that the addition of L-carnitine may prove beneficial for production. Research in neonatal pigs showed that 2 day old pigs reared on a low carnitine, artificial diet had lowered plasma carnitine which suggests that dietary carnitine is necessary for normal carnitine concentrations in the plasma (Coffey et al., 1991). Supplemental dietary carnitine in the weanling pig may improve growth performance. Weeden et al. (1990) found that supplemental carnitine fed from d 14 to d 35 postweaning improved gain:feed in pigs, however, Hoffman et al. (1993) did not find improvement in feed utilization in young pigs with supplemented with L-carnitine. Weeden et al. (1991) and Newton and Haydon (1988) found that the addition of 600 to 1,000 ppm L-carnitine improved average daily gain and average daily feed intake in young pigs. In contrast, Owen et al. (1994b) found no overall effect on growth performance, but did find that pigs on 1,000 ppm L-carnitine were 6% more efficient and 9% heavier on d 35 compared with pigs fed no L-carnitine.

Finishing pigs fed added L-carnitine had better feed efficiency and had less average backfat thickness compared with pigs fed no L-carnitine (Newton and Haydon, 1989; Weeden et al., 1991). Additionally, Owen et al. (1993, 1994b, 1996) reported that lipid accretion was reduced in pigs fed 750 to 1,000 ppm L-carnitine. Higher average daily gains and better feed efficiency were reported in growing-finishing pigs fed 50 ppm L-carnitine (Owen et al., 1994a; Smith et al., 1994). L-carnitine's potential for improving carcass characteristics in finishing pigs was shown by Owen et al. (1993) who found an increase in longissimus muscle area and a reduction in daily lipid accretion rate and a reduction in backfat thickness. An additional study (Owen et al., 1994a) found that 50

ppm L-carnitine was the dosage which provided the optimum response for improving carcass characteristics (i.e. reduced average backfat thickness, tenth rib backfat depth, increased longissimus muscle area, daily CP accretion rate, and improved percentage lean and muscle).

Sows may also benefit from supplementation with L-carnitine. Musser et al. (1997) found that added L-carnitine (50 ppm) decreased feed intake during the first week of lactation and tended to decrease the days to estrus. Similar results were seen by Fremaut et al. (1993) and Harmeyer (1993) in which supplementation with L-carnitine shortened the time between weaning and sows' first estrous, reduced piglet mortality rate, and increased litter weaning weights.

Responses in ruminants to L-carnitine administration have been variable. Supplemental L-carnitine, infused directly either into the rumen or abomasum, was effective in increasing plasma, liver, and milk concentrations of carnitine; however, L-carnitine had little effect on milk yield and composition (LaCount et al., 1995). Dietary, abomasally infused, and intravenous L-carnitine did not affect DM intake, milk yield, and milk composition in dairy cows (Fisher and Erfle, 1974; LaCount et al., 1996a,b). Subcutaneous injections of L-carnitine in dairy cows had no effect on milk or component yields (Staples et al., 1975). However, carnitine degradation did occur in ruminal fluid after 2 weeks of adaptation to dietary carnitine supplementation which may explain why no effects were reported when L-carnitine was added as a supplement (LaCount et al., 1996a). Yavuz et al. (1997) reported that supplemental L-carnitine reduced plasma urea nitrogen concentrations in Holstein calves fed diets containing 50% broiler litter. This

suggests that L-carnitine may be influencing nitrogen metabolism (Yavuz et al., 1997). Supplemental L-carnitine also improved growth in grazing beef calves fed liquid supplements containing urea and was associated with reduced ruminal ammonia nitrogen levels (White et al., 1997). A study using heifers (Hill et al., 1995) showed that dietary L-carnitine reduced marbling score, but little to no consistent effects have been found in steers (Hill et al., 1994).

Potential in preventing hyperammonemia. L-carnitine may provide a practical means of preventing hyperammonemia in ruminants. The administration of L-carnitine in mice has been shown to provide protection against the effects of known lethal levels of ammonium acetate (Grisolia et al., 1984; Matsuoka et al., 1991; Matsuoka and Igisu, 1993) and against the toxicity of ammonia induced by prolonged use of sodium benzoate (O'Connor et al., 1987; Michalak and Qureshi, 1990; Ratnakumari et al., 1993). Igisu et al. (1995) found that L-carnitine lowered the concentration of ammonia in both blood and in the brain during ammonia intoxication. Chapa et al. (1997) observed lowered plasma ammonia nitrogen in sheep administered an intravenous L-carnitine solution 30 minutes prior to an oral urea load test. However, other studies have not shown the protective effect of L-carnitine against ammonia toxicity (Deshmukh and Rusk, 1988; Deshmukh et al., 1990; Morris et al., 1998).

There are several hypotheses that have been proposed that explain how L-carnitine may afford protection against toxicity. One possibility is that L-carnitine may be stabilizing liver mitochondrial membranes by removing long-chain acyl CoA from the mitochondrial membrane and/or increasing the respiratory activity of the mitochondria,

thereby improving the structural and metabolic integrity of the mitochondria (Di Lisa et al., 1985; Bellei et al., 1989). In contrast, Kloiber et al. (1988) suggested that the effectiveness of L-carnitine may be due to osmoregulation. L-carnitine resembles, in structure, other substances which have been described to be "osmoprotectants" (Kloiber et al., 1988). When L-carnitine was tested along with other osmoprotectants (i.e. choline, betaine, and TMAO), all the compounds tested presented some degree of protection against induced ammonia toxicity (Kloiber et al., 1988). The suggested mechanism is that enzymes or other proteins are stabilized in the presence of the osmoprotectants (Kloiber et al., 1988).

Another possible hypothesis of L-carnitine's mode of action in preventing acute ammonia intoxication is through the enhanced incorporation of ammonia into urea (O'Connor et al., 1987). O'Connor et al. (1987) observed that mice treated with L-carnitine (16 mmol/kg BW) experienced a continuous rise in blood urea nitrogen until a plateau was reached 1 h after injection, whereas mice not treated with L-carnitine experienced a rise in blood urea nitrogen, but died within 15 min of ammonium acetate administration. O'Connor et al. (1987) suggests that stimulation of urea synthesis by L-carnitine occurs by increasing β-hydroxybutyrate concentration, and therefore the $NADH/NAD^+$ ratio. Meijer et al. (1975) demonstrated that the addition of β-hydroxybutyrate to hepatocytes incubated in ammonium chloride stimulated the synthesis of urea. The conclusion was that the stimulatory effect resulted from increasing the reducing equivalents and enhancing ATP generation within the mitochondria (Meijer et al., 1975). The route of administration may influence the effectiveness of L-carnitine.

O'Connor et al. (1986) found that, in mice, intraperitoneal injections offered the most protection followed by intravenous and intramuscular administrations, with subcutaneous administration of L-carnitine offering the least amount of protection against acute ammonia intoxication. In ruminants, the microbial population of the rumen may determine to what extent L-carnitine is degraded in the rumen and absorbed post-ruminally. LaCount et al. (1996a) found that carnitine degradation was greater in ruminal fluid from cows that had adapted to dietary carnitine supplementation for 2 weeks.

Chapter 3

Supplemental Dietary Protein Effects on Selected Plasma Energy and Nitrogen Metabolites and Insulin of Grazing Dairy Cows

Introduction

Annual ryegrass (*Lolium multiflorum*) is a high quality forage widely grown as temporary pasture throughout the southeastern region. Ryegrass is typically grazed from November until May and is used to support dairy production through the winter months (Eichhorn et al., 1996). Well-fertilized ryegrass usually contains 20-30% CP and of this 75% may be rapidly degraded in the rumen, especially in immature pastures (Eichhorn et al., 1996). In addition to grazing, dairy cows across the region may also be fed grain supplements, which typically contain 18-20% CP (air-dry basis), to offset any negative effects on milk production that may occur when pasture availability is low. Dairy cows that are supplemented in combination with ryegrass grazing may be consuming diets containing 22-25% CP which is substantially higher than required for Holsteins in early lactation (NRC, 1988). Excess protein and insufficient energy have been linked to suboptimal performance of livestock (Short and Adams, 1988; Zavy and Geisert, 1994). Examples of subptimal performance due to excess protein include decreased fertility (Wilson et al., 1985; Elrod and Butler, 1991), increased embryo mortality (Zavy and Geisert, 1994), and increased number of days open (Sonderegger and Schurch, 1977). Examples of suboptimal performance due to insufficient energy include delayed puberty, lengthened postpartum anestrus and onset of anestrus in estrus cows or heifers (Short and Adams, 1988). A subclinical form of ammonia toxicity, causing aberrations in the animal's

metabolism resulting in reduced hepatic glucose production (Spires and Clark, 1979; Fernandez et al., 1990a), reduced glucose utilization by insulin-sensitive tissues (Sener et al., 1978; Fernandez et al., 1988, 1990a,b) and derangements in hormone action (Jordan et al., 1983) may result from excess protein which is rapidly hydrolyzed to ammonia in the rumen. Because most of the CP from ryegrass may be degraded in the rumen, it is important to determine the maximum tolerable protein concentrations in grain supplements that will maximize milk production yet maintain plasma urea nitrogen levels below harmful levels (< 20 mg/dL) (Ferguson et al., 1988, 1993). The addition of rumen undegradable protein, which is a slowly degraded protein, may not only alleviate some of the reproductive problems associated with high soluble nitrogen, but previous research suggests that milk yield in the early lactation cow increases in response to feeding this type of protein (Armstrong et al., 1990; Burke et al., 1997; Rodriguez et al., 1997). The objectives of this study were twofold. The first objective was to monitor the metabolic status of cows grazing ryegrass pastures in relation to nitrogen and energy status through the measurement of key blood metabolites. The second was to determine the effect of dietary nitrogen source and concentration in grain supplements that will maximize milk yield and maintain plasma urea nitrogen levels below dangerous levels (< 20 mg/dL) for cows on all ryegrass forage.

Materials and Methods

Animal care and sampling. A different set of cows was used for each year and each set of cows was reproductively sound at the start of the experiment. Year 1 (YR1) data were collected using sixty-four mature Holstein cows from October 1995 until May

1996. Year 2 (YR2) data were collected using sixty mature Holstein cows from October 1996 until May 1997. For both years, all cows were blocked on the basis of milk equivalents and calving group and were randomly assigned to one of three protein supplement treatments. Calving groups were further divided into two groups: Partum and Post-partum. The Partum group was defined as those cows in which the protein supplements and grazing were imposed at calving (avg days post-partum = 0) and in the Post-partum group the protein supplements and grazing were imposed after calving (avg days post-partum = 41). Prior to the start of the experiment, Partum cows were fed a corn silage-based PMR and the Post-partum cows were fed a TMR. Grazing was not initiated until the start of the experiment. All cows were bred A.I. to a single sire and breeding was conducted by one technician twelve hours after the observation of estrus. Cows were milked twice daily and body condition was scored (five-point scale; 1= thin to 5 = fat) every ten days. Blood for YR1 was collected at 50 and 100 days post-partum during the morning milking and on breeding days at insemination. Based on the results of YR1, blood samples for YR2 were collected, during the morning milking, at 50, 100, and 150 days post-partum in order to follow the cows into positive energy balance and also on breeding days. All blood was collected via the coccygeal vein into 7-mL vacutainer tubes containing potassium oxalate and sodium fluoride (Monoject Blood Collection Tubes; Sherwood Medical, St. Louis, MO).

Protein supplements and pasture management. Ingredient composition for the protein supplements for both YR1 and YR2 were identical (Table 3.1). All three protein supplements were formulated to meet or exceed the NRC requirements for CP when fed

Table 3.1. Composition of protein supplements for Year 1 and Year 2.

	Protein Supplements[a,b]		
	Suppl 1 125% CP 110% UIP	Suppl 2 100% CP 90% UIP	Suppl 3 100%CP 100% UIP
Ingredient:			
Corn, ground	32.8	42.8	43.6
Soybean meal	18.4	8.4	--
Corn gluten meal	--	--	4.1
Blood meal	--	--	1.3
Molasses	--	--	2.2
Minerals[c]	2.2	2.2	2.2
Ryegrass	43.6	43.6	43.6
Bermudagrass	3.3	3.3	3.3
Total	100.0	100.0	100.0
Chemical Composition:			
DM	25.2	25.2	25.1
Protein	23.1	19.1	19.2
UIP	8.1	6.1	7.2
ADF	14.3	13.6	12.9
NDF	24.8	24.4	24.2
NE_l, Mcal	79.4	79.8	79.7
Ca	.77	.74	.75
P	.45	.44	.43

[a] Expressed as % of NRC requirements for early-lactation cows on a DM basis.
[b] Based on a 612 kg cow producing 45 kg 3.5% FCM daily and consuming 4% body weight in DM.
[c] Minerals were supplemented in a custom mix containing: calcium carbonate (27%), deflourinated rock phosphate (6%), salt (23%0), magnesium carbonate (3%), manganese oxide (.20%), zinc oxide (.15%), ferrous carbonate (.10%), copper sulfate (.035%), cobalt carbonate (.0006%), calcium iodate (.01%), sodium selenite (.0023%), vitamin A (2640 IU/kg diet), vitamin D3 (1320 IU/kg diet), vitamin E (3 IU/kg diet).

in combination with the 24% protein from ryegrass for the early lactation Holstein cow. Supplement 1 (Control; Suppl 1), was a corn/SBM based supplement which contained excess levels of CP (22.2% CP) and degradable intake protein (10.2% DIP). Supplement 2 (Suppl 2) was a corn/SBM based supplement which met NRC requirements for CP (15.6% CP) and DIP but was formulated to be deficient in UIP. (7.9% UIP). Supplement 3 (Suppl 3) was a corn/corn gluten meal/blood meal based supplement which met NRC requirements for the CP (15.6% CP) DIP, and UIP (9.0% UIP) fractions for early lactation dairy cows. All cows were individually fed the supplements prior to the a.m. and p.m. milking and refusals were recorded daily. Cows were fed according to milk production at a .5:1.4 ratio of kg of supplement per kg of milk. All cows grazed a common pasture for 24 to 48 hours and then rotated to a new pasture. Ryegrass/oats (for early fall grazing) and ryegrass (for winter and spring grazing) was no-till planted in the fall of each year and stocked at a rate of one cow per .4 ha. Pasture availability and quality were determined bimonthly and pasture excess was removed by clipping. Bermudagrass hay and water were available ad libitum.

Sample Handling and Analytical Procedures. During both years, blood samples were collected during the morning milking, placed in an ice bath, and transported to the laboratory. Blood was centrifuged and the plasma was harvested, divided into two equal aliquots, placed in storage tubes, and frozen at -20° C until analyses. The daily samples collected for YR1 were analyzed for plasma ammonia nitrogen and urea nitrogen (Laborde et al., 1995), glucose (Method No. 315; Sigma Chemical Co., St. Louis, MO), NEFA (NEFA-C Kit, ACS-ACOD Method; Wako Chemicals USA, Inc., Richmond, VA),

and β-hydroxybutyrate (BOHB; Method No. 310-A ; Sigma Chemical Co.). Additionally, glycated hemoglobin (Method No. 442-A; Sigma Chemical Co.) was analyzed on Day 100 as an indicator of long-term carbohydrate metabolism. Blood from samples collected at day of breeding were analyzed for plasma glucose, NEFA, urea nitrogen, and insulin. Samples collected for YR2 were analyzed for the same metabolites and hormones; however, glycated hemoglobin was not analyzed for the Day 100 samples. All plasma metabolite concentrations were determined using spectrophotometric methods. Samples were assayed in duplicate, and measurements resulting in a ≥5% error in precision were reanalyzed. Plasma insulin was analyzed using a double antibody radioimmunoassay (Bunting et al., 1994). The mean intraassay and interassay CV for the insulin radioimmunoassay were 5.3% and 4.5%, respectively. Samples were assayed in duplicate, and measurements resulting in a ≥5% error in precision were reanalyzed.

Statistical Analyses. The experimental analyses for both years was a randomized completed block design with a factorial treatment arragement. The three factors included two milk equivalent blocks, two types of calving groups, and three protein supplements. Individual cow was the experimental unit. All data were analyzed using the MIXED procedure of SAS (1992). Only breedings 1 - 3 were used in the analyses of the samples collected at breeding, since the number of breedings per cow ranged from 1 to 6. Correlations for treatment, day, and number of breedings were also computed. Data for both years were analyzed independently and then combined to determine whether there was an effect of year.

Results

Year 1. Body condition score was lowest ($P < .0001$) in the Partum group compared with the Post-partum group (2.3 vs 2.6; Figure 3.1). Body condition was also affected ($P < .04$) by protein supplementation with cows fed Suppl 2 showing the lowest BCS (Figure 3.2). Cows in the Partum group had lower BCS compared with Post-partum cows across all supplements, however, BCS was lowest for the Partum cows fed Suppl 2 (Calving group x Suppl, $P<.04$; Figure 3.3). The effects of protein supplements on plasma metabolites and insulin are listed in Table 3.2. Plasma urea nitrogen was higher ($P < .0001$) in cows fed Suppl 1 compared to Suppl 2 and 3β (15.07 ± 0.42 vs 12.36 ± 0.48 vs 12.85 ± 0.48 mmol/L; Figure 3.4). Plasma glycated hemoglobin, a long-term indicator of glucose metabolism, was not affected ($P > .10$) by the protein supplement or calving group. Additionally, glucose, BOHB, NEFA, ammonia nitrogen, and insulin were not affected ($P > .10$) by the protein supplement. Plasma NEFA concentration was higher ($P < .0001$) on Day 50 vs Day 100 (255.1 ± 22.9 vs 136.5 ± 6.1 μEq/L; Figure 3.5). Plasma insulin levels were higher ($P < .08$) on Day 100 vs Day 50 (18.5 ± 2.1 vs 14.0 ± 1.6 μU/mL). Plasma BOHB, glucose, urea nitrogen, and ammonia nitrogen were not affected ($P > .10$) by days post-partum. At time of breeding, plasma urea nitrogen was higher ($P < .0004$) in Suppl 1 compared with Suppl 2 and 3 (14.31 ± 0.37 vs 11.71 ± 0.54 vs 12.24 ± 0.42 mmol/L). Plasma NEFA levels in the Post-partum group were higher during the first breeding and then dropped by the second and third breedings; however, the Partum group showed the highest NEFA values during the second breeding

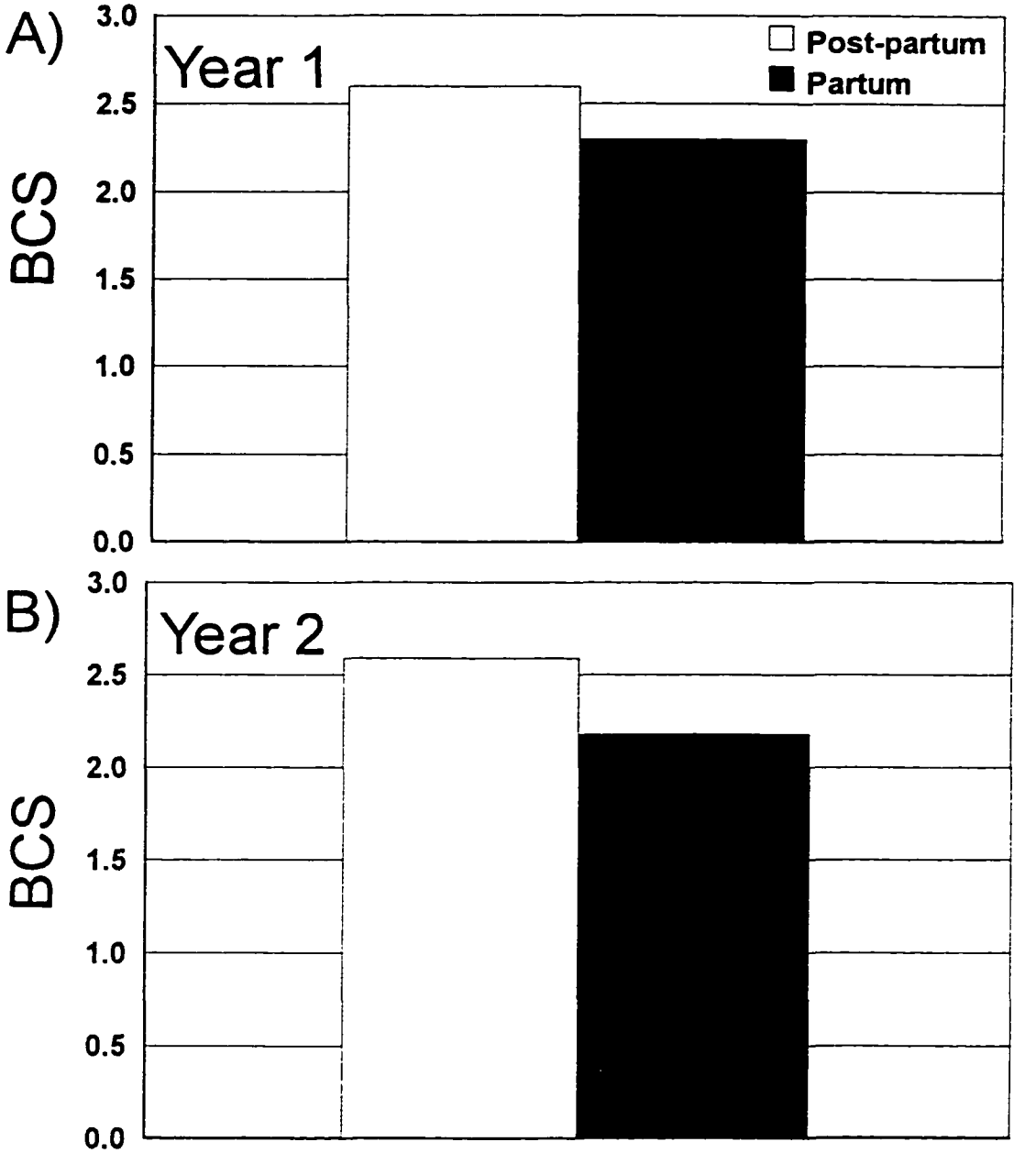

Figure 3.1. Changes in body condition score for grazing dairy cows in A) Year 1 and B) Year 2 in response to calving group.

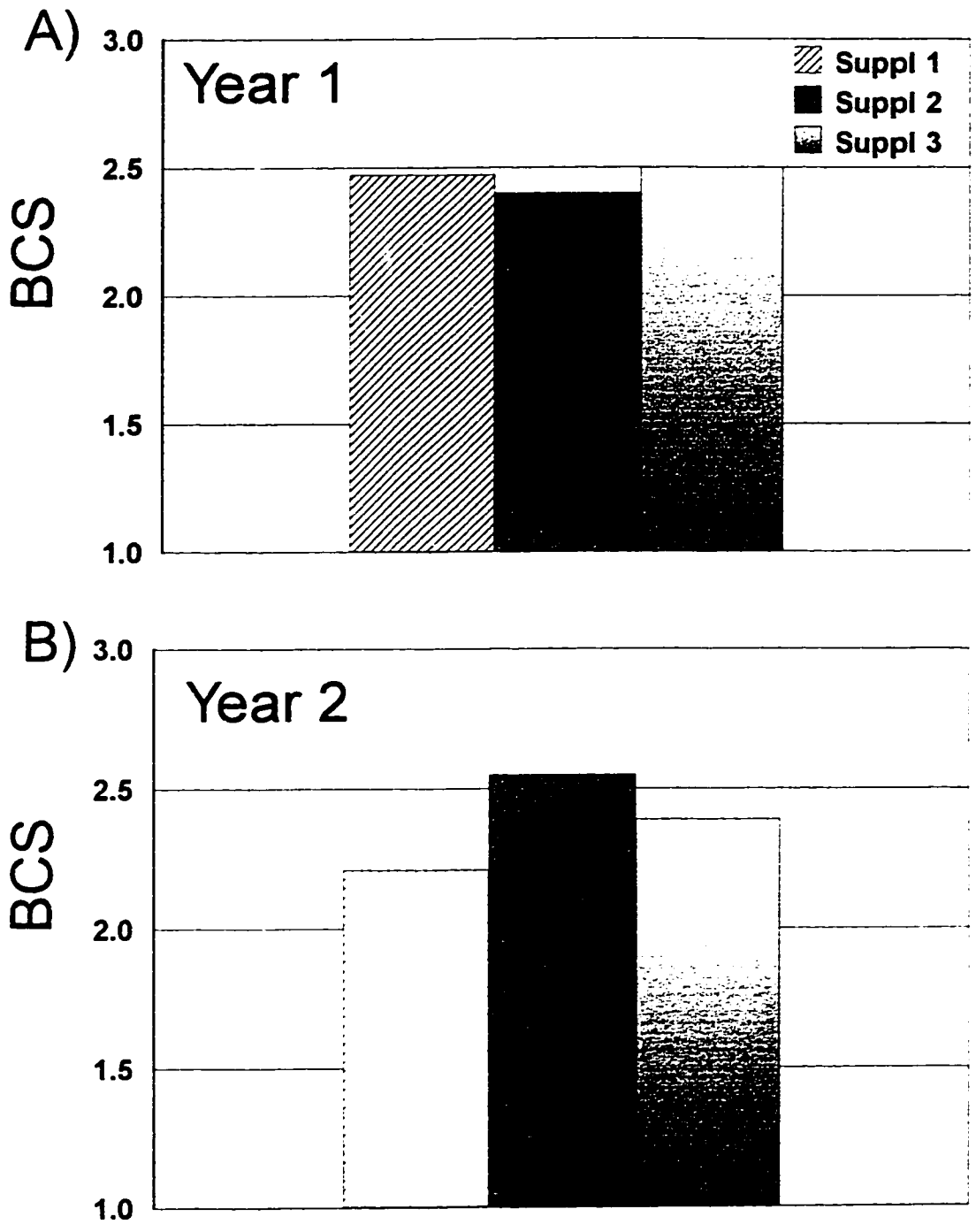

Figure 3.2. Changes in body condition score for grazing dairy cows during A) Year 1 and B) Year 2 in response to protein supplementation.

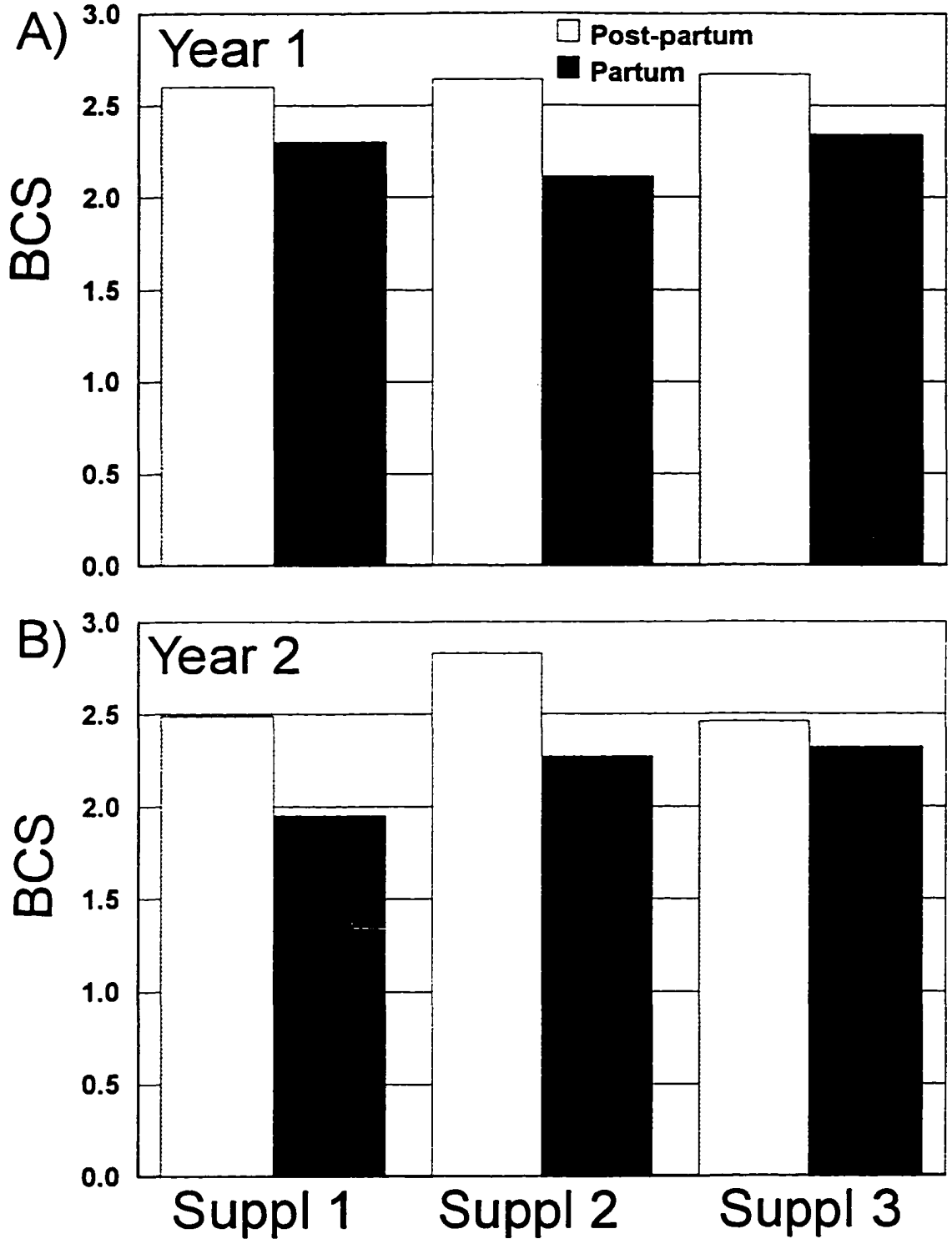

Figure 3.3. Effect of calving group and protein supplementation during A) Year 1 and B) Year 2 on body condition score for grazing dairy cows.

Table 3.2. Effect of protein supplements on plasma metabolites and insulin by individual years and combination of Year 1 and 2.

	Protein Supplement[a]				P-value	
Metabolite	Suppl 1	Suppl 2	Suppl 3	SEM	Suppl	Year
Year 1						
Ammonia N, μmol/L	78.0	70.4	76.9	5.6	NS	-
Urea N,mmol/L	15.07[x]	12.36[y]	12.94[y]	.4	.0001	-
Glucose, mmol/L	3.59	3.44	3.58	.10	NS	-
NEFA, μEq/L	192.4	209.8	215.1	14.0	NS	-
BOHB, mmol/L	.57	.53	.70	.09	NS	-
Insulin, μU/mL	21.17	21.11	17.55	2.4	NS	-
Year 2						
Ammonia N, μmol/L	82.9	80.1	77.1	2.0	NS	-
Urea N, mmol/L	16.49[x]	12.16[y]	13.27[y]	.3	.0001	-
Glucose, mmol/L	3.27	3.35	3.26	.04	NS	-
NEFA, μEq/L	140.0	159.8	166.5	9.0	NS	-
BOHB, mmol/L	.54	.57	.49	.04	NS	-
Insulin, μU/mL	27.74[x]	39.20[y]	31.11[x]	2.5	.008	-
Year 1&2						
Ammonia N, μmol/L	82.2	76.4	76.7	3.3	NS	NS
Urea N, mmol/L	16.13	12.33	13.28	.4	NS	NS
Glucose, mmol/L	3.44	3.42	3.42	.05	NS	NS
NEFA, μEq/L	174.5	193.7	213.6	11.6	NS	NS
BOHB, mmol/L	.56	.57	.59	.05	NS	NS
Insulin, μU/mL	25.34	31.17	25.97	2.4	NS	NS

[a] Protein supplements were Suppl 1 = 125% crude protein, 110% undegradable intake protein; Suppl 2 = 100% crude protein, 90% undegradable intake protein; Suppl 3 = 100% crude protein, 100% undegradable intake protein. Expressed as % of NRC requirements for early-lactation cows on a dry matter basis.

[xy] Within a row, means lacking a common superscript letter differ.

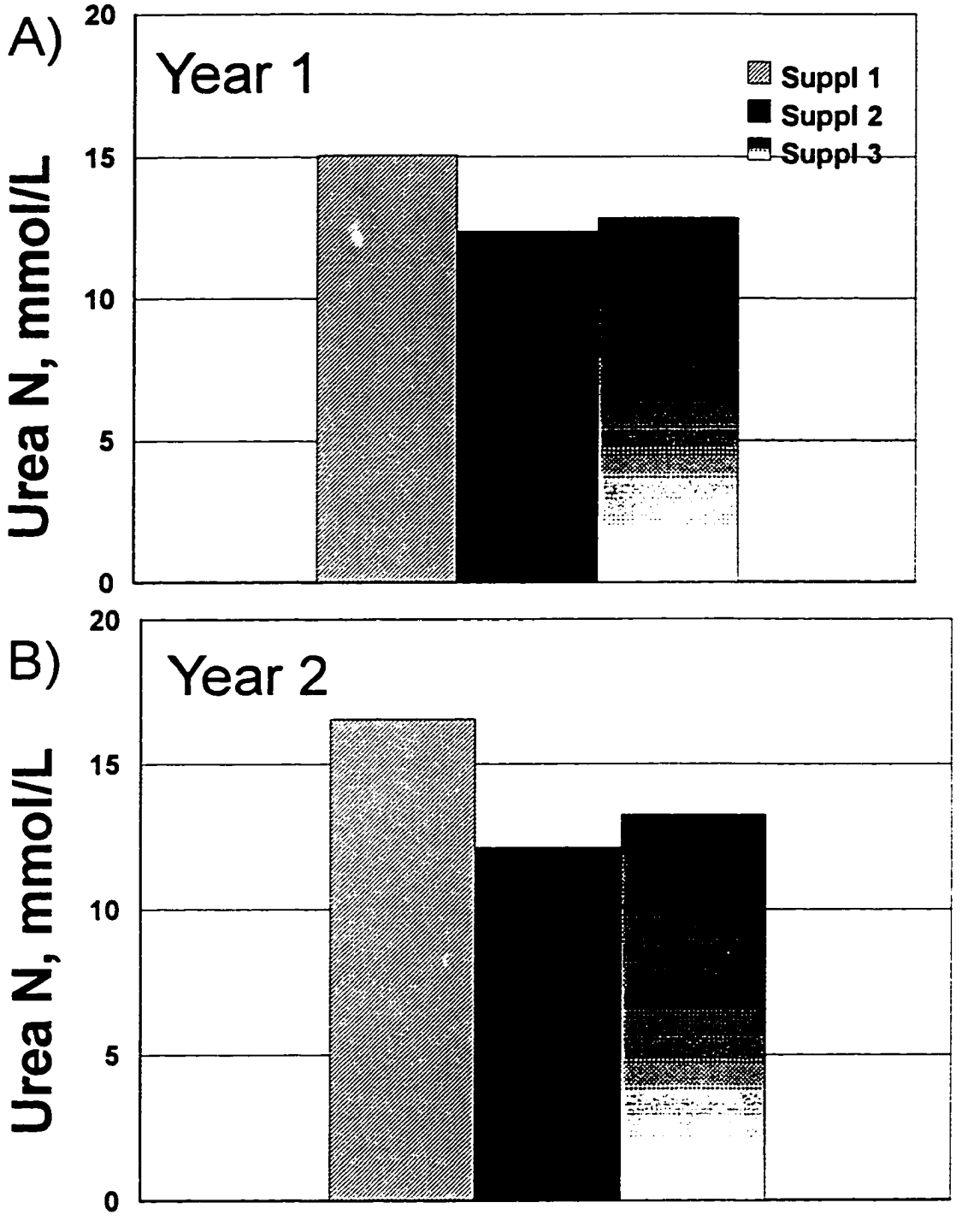

Figure 3.4. Changes in plasma urea N concentration in grazing dairy cows in response to protein supplementation for A) Year 1 and B) Year 2.

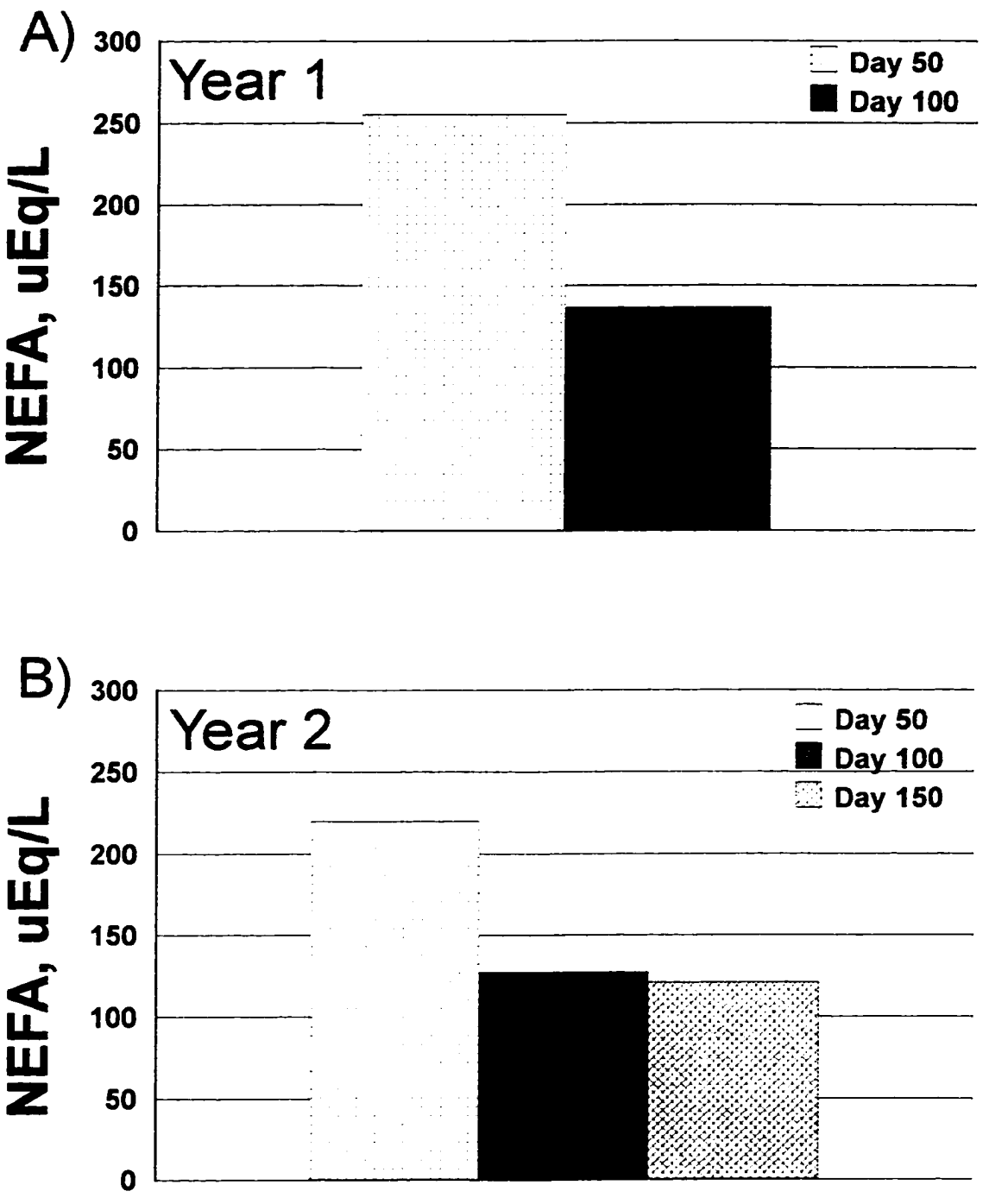

Figure 3.5. Effect of days post-partum on plasma NEFA concentrations in grazing dairy cows during A) Year 1 and B) Year 2.

(Calving group x Breeding, P< .03). The effect of calving group on plasma metabolites and insulin is listed in Table 3.3. Plasma ammonia nitrogen concentrations were higher (P < .004) in the Post-partum group compared with the Partum group (85.0 ± 6.0 vs 65.5 ± 2.5 μmol/L; Figure 3.6). The Post-partum group had higher plasma glucose (3.66 ± 0.06 vs 3.37 ± 0.09 mmol/L; P < .05) and higher plasma insulin (22.7 ± 1.9 vs 17.4 ± 2.1 μU/mL; P < .06) compared with the Partum group. Calving group did not affect (P > .10) plasma urea nitrogen or BOHB concentrations.

Year 2. Body condition score was also lowest (P<.0001) in the Partum group of cows compared with the Post-partum cows. Body condition score was lowest (P<.0001) in cows fed Suppl 1 compared with cows fed the other protein supplements (Figure 3.2). Cows in the Partum group had lower BCS compared with Post-partum cows across all supplements, however, BCS was highest for the Post-partum cows fed Suppl 2 (Calving group x Suppl, P<.0001; Figure 3.3). The effect of protein supplements on plasma metabolites and insulin is listed in Table 3.2. Urea nitrogen was affected by treatment (P< .0001) with cows in Suppl 1 having the highest concentrations compared to cows in Suppl 2 and 3 (16.49 ± 0.37 vs 12.16 ± 0.33 and 13.27 ± 0.33 mmol/L, respectively; Figure 3.4). Plasma glucose concentrations were not affected (P > .10) by calving group, supplement, or day. Plasma insulin levels were highest (P < .008) in Suppl 2 compared with Suppl 1 and 3 (39.2 ± 2.5 vs 27.7 ± 2.6 and 31.1 ± 2.5 μU/mL, respectively). Plasma BOHB concentrations were highest (P < .02) at Day 100 compared to Day 50 and 150 (0.61 ± 0.05 vs 0.47 ± 0.25 vs 0.50 ± 0.02 mmol/L, respectively). Plasma NEFA were higher (P = .0001) on Day 50 compared with Day 100 and 150

Table 3.3. Effect of calving group on plasma metabolites and insulin by individual year and combination of Year 1 and 2.

Metabolite	Calving Group[a]		SEM	P-value	
	Partum	Post-partum		Cgroup	Year
Year 1					
Ammonia N, μmol/L	65.3[x]	85.0[y]	5.2	.004	-
Urea N,mmol/L	13.58	13.36	.57	NS	-
Glucose, mmol/L	3.44[x]	3.65[y]	.09	.05	-
NEFA, μEq/L	191.3	215.2	18.65	NS	-
BOHB, mmol/L	.55	.66	.10	NS	-
Insulin, μU/mL	25.70[x]	18.32[y]	3.60	.06	-
Year 2					
Ammonia N, μmol/L	80.1	83.4	5.5	NS	-
Urea N,mmol/L	13.63[x]	15.14[y]	.60	.04	-
Glucose, mmol/L	3.24	3.38	.05	NS	-
NEFA, μEq/L	148.7[x]	198.3[y]	19.34	.0001	-
BOHB, mmol/L	.51	.59	.10	NS	-
Insulin, μU/mL	25.75[x]	40.20[y]	3.93	.0001	-
Year 1&2					
Ammonia N, μmol/L	72.8	84.1	3.8	NS	NS
Urea N, mmol/L	13.59	14.24	.41	NS	NS
Glucose, mmol/L	3.34	3.52	.06	NS	NS
NEFA, μEq/L	170.0	217.9	13.44	NS	NS
BOHB, mmol/L	.53	.62	.06	NS	NS
Insulin, μU/mL	25.73	29.26	2.71	NS	NS

[a] Calving groups were Partum = cows in which the protein supplements and grazing were imposed at calving (avg days post-partum = 0); Post-partum = cows in whith the protein supplements and grazing were imposed after calving (avg days post-partum = 41).
[xy] Within a row, means lacking a common superscript letter differ.

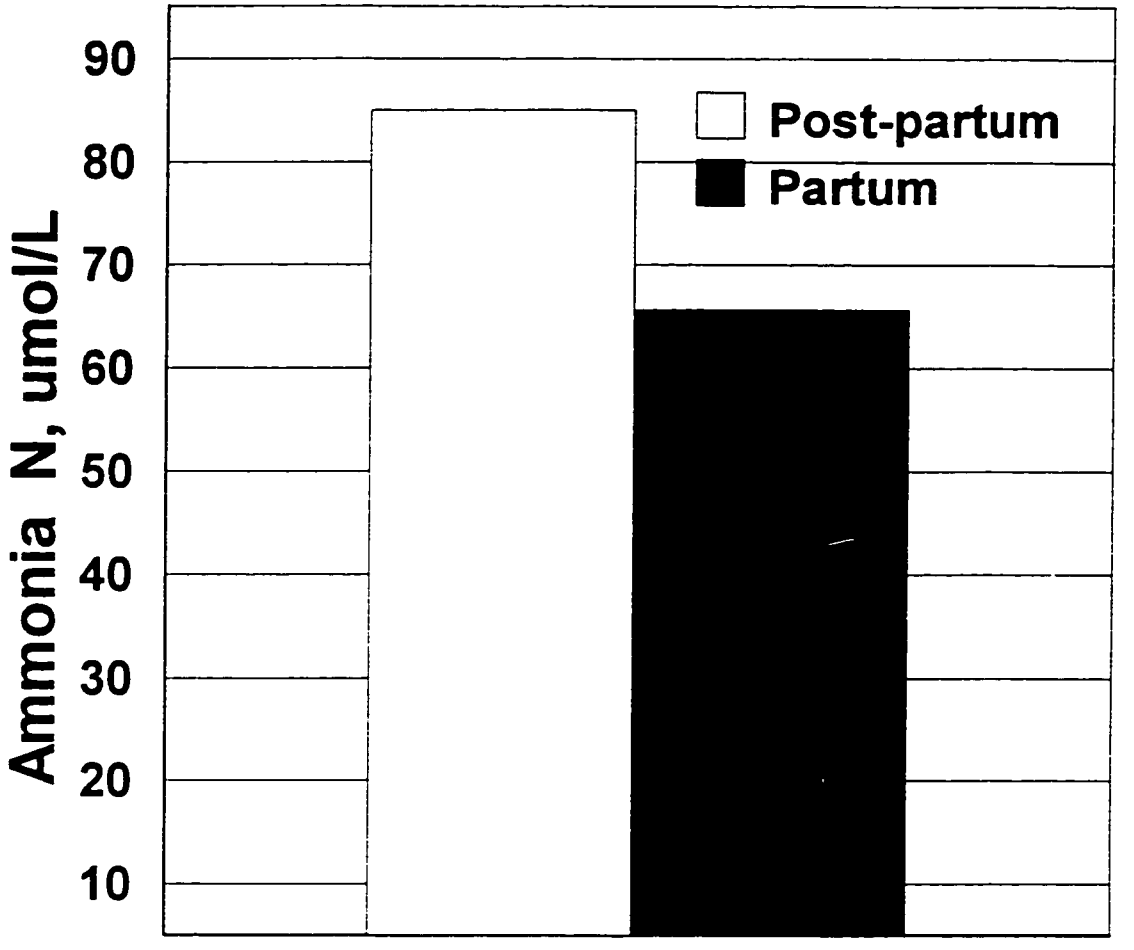

Figure 3.6. Effect of calving group on plasma ammonia N concentrations in grazing dairy cows during Year 1.

(219.6 ± 14.2 vs 127.4 ± 6.46 vs 121.2 ± 4.23 μEq/L, respectively; Figure 3.5). Plasma urea nitrogen levels were higher (14.87 ± 0.34 vs 13.94 ± 0.34 vs 13.12 ± 0.35 mmol/L; P< .003) and plasma ammonia nitrogen concentrations were higher (87.1 ± 2.9 vs 75.7 ± 1.9 and 76.5 ± 1.3 μmol/L; P < .0003) at Day 50 compared with Day 100 and 150 (Figure 3.7). At the time of breeding, cows fed Suppl 3 had lower (P < .05) plasma glucose values compared to Suppl 2 and 1 (3.38 vs 3.51 and 3.46 mmol/L, respectively). At the time of breeding, cattle in the Post-partum calving group had higher (P < .05) NEFA values compared to cattle in the Partum calving group (256.1 ± 16.2 vs 209.2 ± 15.6 μEq/L). At breeding, plasma urea nitrogen values were also higher (P < .02) for cows fed Suppl 1 compared with cows fed the other two supplements (17.20 ± 0.6 vs 13.85 ± 0.7 and 13.76 ± 0.9 mmol/L). The effect of calving group on plasma metabolites and insulin is listed in Table 3.3. Cows in the Post-partum calving group had higher (P < .05) NEFA and urea nitrogen values compared with the Partum group (176.6 ±10.0 vs 135.6 ± 7.4 μEq/L; 14.35 ± 0.4 vs 13.33 ± 0.3 mmol/L, respectively). Cows showed higher (P < .005) NEFA values during the first breeding compared to the second and third breedings (270.5 ± 15.2 vs 191.0 ± 20.2 and 196.3 ± 26.9 μEq/L, respectively).

Discussion

Excess dietary protein has been shown to inhibit reproductive efficiency in dairy cows (Folman et al., 1983; Ferguson et al., 1993; McCormick et al., 1994). A depression in pregnancy rates and an increase in embryo mortality has been attributed to lower fertility due to high plasma urea nitrogen and ammonia nitrogen concentrations in cows consuming excess protein (Jordan and Swanson, 1979; Elrod and Butler,1993;

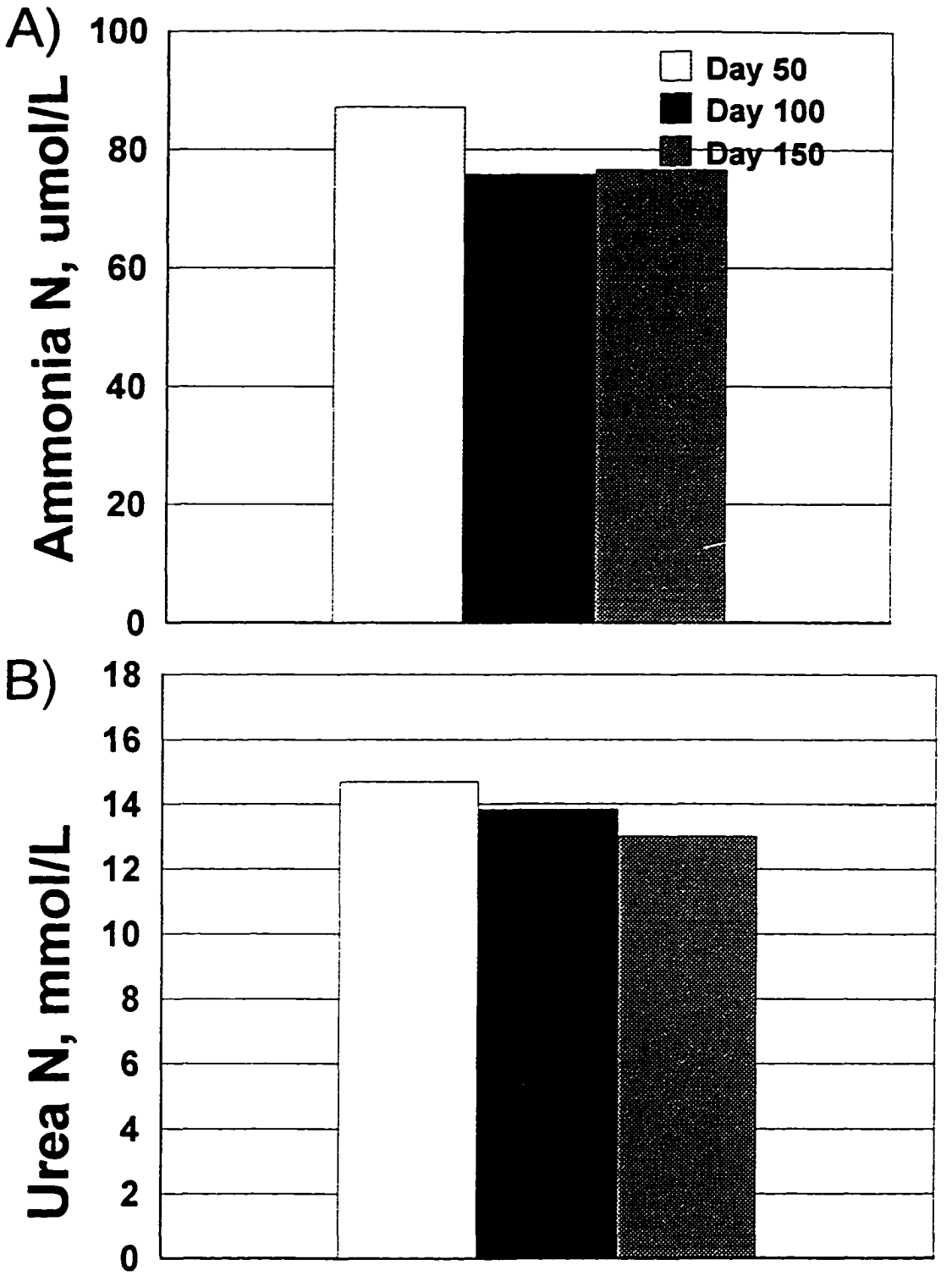

Figure 3.7. Effect of days post-partum on A) plasma ammonia N and B) plasma urea N for grazing dairy cows during Year 2.

McCormick et al., 1994). Previous research at the Southeast Research Station in Louisiana demonstrated that cows fed a 20% (as-fed) protein grain mix while on ryegrass pasture experienced a 41% depression of first service conception rate and a 29% reduction in overall conception rate compared to cows fed protein at recommended concentrations (McCormick et al., 1994). Support is growing for a mechanism which does not implicate the direct effects of plasma ammonia nitrogen or urea nitrogen, but to an interaction between the level of protein and energy in the diet (Sonderegger and Schurch, 1977; Ferguson et al., 1988; Carroll et al., 1994; Garcia-Bojalil et al., 1994). Several studies have shown that the ratio between protein and energy intake might be more important than either protein or energy intake alone (Sonderegger and Schurch, 1977; Hammond, 1983; Ferguson et al., 1988). Other studies have shown no negative reproductive responses in cows fed excess protein. In these studies, the cows lost little body condition (Carroll et al., 1994), were nonlactating (Garcia-Bojalil et al., 1994), or were producing milk at a moderately low level (Howard et al., 1987). As expected, the plasma urea nitrogen concentration for both YR1 and YR2 was highest in cows fed Suppl 1 which was composed of excess total protein. The protein would have been degraded in the rumen and the ammonia produced through rumen microbial fermentation would have been converted to urea in the liver (Visek, 1984). Plasma urea nitrogen concentrations appear to be influenced by several dietary factors including protein intake, energy intake, and level of feeding (Hammond, 1983). Increased protein intake results in protein wastage because the excess is excreted as urea in the urine, whereas reduced energy intake permits elevated use of protein for energy; both situations elevate plasma urea nitrogen

concentrations (Hammond, 1983). Studies in ruminants (Preston et al., 1965; Hammond, 1983), horses (Fonnesbeck and Symons, 1969; Patterson et al., 1985), and other nonruminants (Eggum, 1970) have shown that plasma urea nitrogen increases as dietary protein increases.

Calving group (Partum vs. Post-partum) seemed to exert a great effect on the response of the dairy cows. Cows in the Partum group had significantly lower BCS in both YR1 and YR2. During both years, all cows placed on the study began with similar BCS (2.8 ± .30), this decrease indicates that these cows were in a greater nutritional stress and depleted body condition to meet the demands of production. In this study, BCS was affected by both protein supplementation and calving group. The BCS was consistently lower in the Partum group cows regardless of protein supplement and cows fed Suppl 1 showed a mean score below 2.0 during YR2. Again, this suggests that these cows were not meeting their energy demands. This could possibly be due to the cows not being able to consume enough pasture to meet their energy needs for high milk production and therefore depleted body stores of fat to meet milk production. Milk production in this study was affected by calving group. Partum cows had higher 3.5% FCM compared with Post-partum cows for YR1 (36.4 ±.90 vs 29.7 ± .90 kg, respectively) and YR2 (31.8 ± .90 vs 28.6 ± .30 kg, respectively). A grazing study conducted by Holden et al. (1994) showed that pasture DMI ranged from 11.5 to 15.6 kg/hd/d depending on season and forage quality. During early spring, the estimated NE_L intakes were below NRC requirements. Holden et al. (1994) suggested that the rapid rate of passage and the low DM content of the lush spring pasture prevented the cows from consuming enough

pasture DM to meet the energy needs for high levels of milk production. A similar situation may occur in Louisiana during late fall when early lactation cows are grazing immature ryegrass pastures. Low condition scores, evident in this study, may be related to the higher maintenance requirements of grazing cows compared with cows fed a TMR or those that are confined (Caton and Dhuyvetter, 1997). In the case of grazing ruminants, energy utilized by muscle tissue, which is approximately 23% of the total energy for maintenance, may be larger depending on the amount of work required for forage acquisition and processing (Ferrell, 1988; Caton and Dhuyvetter, 1997). Data from grazing sheep indicate that energy expenditure can be 30% greater than that of confined sheep (Osuji, 1974).

During both years, the cows on this study exhibited characteristic metabolic responses in regard to a normal lactation curve. As the cows progressed from 50 days post-partum toward 100 and 150 days post-partum, the metabolic parameters reflected the changing physiological state from negative energy balance to positive energy balance. Plasma NEFA levels which were highest at 50 days post-partum decreased by 100 and 150 days post-partum and BOHB levels were highest at 100 days post-partum which suggests that early lactation cows were mobilizing body tissues in order to meet the demands of production (NRC, 1988). However, as cows moved past peak lactation, NEFA levels may have dropped in response to an increase in feed intake (NRC, 1988). The response of plasma insulin reflects the metabolic changes due to mobilization of body tissues for energy. An increase in plasma NEFA levels in early lactation was probably caused by a decrease in plasma insulin concentrations because insulin inhibits lipolysis

(Murray,1990; Amaral-Phillips et al., 1993). Additionally, a decrease was seen in plasma urea nitrogen and ammonia nitrogen. This decrease by 100 and 150 days post-partum could be a reflection of passing peak lactation and increasing DMI and/or the maturation of the pasture in which less soluble nitrogen was available. These decreases in plasma urea and ammonia occurred regardless of protein supplement. However, calving group seemed to have more of an effect on the extent of the response. Partum cows and Post-partum cows responded differently during early lactation. At 50 days post-partum, the Post-partum cows had higher plasma glucose, ammonia nitrogen, and urea nitrogen concentrations; however, by 100 days post-partum, the plasma glucose, ammonia nitrogen, and urea nitrogen concentrations had decreased. This was not true for the Partum cows which had consistently similar values from 50 to 150 days post-partum. A possible explanation could be a reflection of the low BCS. Because the Partum cows had lower energy reserves compared with the Post-partum cows, the carbon skeletons of the excess amino acids are possibly being used as an energy source during early lactation (Tyrrell et al., 1970). After peak lactation, the Partum cows then had similar values of plasma urea nitrogen and ammonia nitrogen compared with the Post-partum cows. In summary, Suppl 1, that supplied excess total protein, exerted negative effects on body condition and plasma nitrogen metabolites but had a minimal effect on plasma energy metabolites and insulin. The nutritional status of the cows post-partum and prior to grazing seemed to have a greater effect than did dietary protein supplements on the ability of the cow to maintain condition during early lactation.

Implications

The nutritional status of the dairy cows prior to grazing seemed to have a great effect on the ability of the cow to maintain condition during early lactation. All cows showed responses to the days post-partum following a normal lactation and DM intake curve regardless of protein supplementation and calving group. However, the composition of the protein supplements exerted the greatest effects on body condition and nitrogen status but had a minimal effect on other metabolic parameters. Body condition scores were lowest in the cows fed excess protein. It is possible that if the energy content of the supplements were increased, that the negative effects associated with calving day and cattle on ryegrass pastures could be alleviated. However, more research is warranted to develop a supplement that would increase milk production and fertility in early lactation dairy cows grazing ryegrass.

Chapter 4

Influence of Intravenous L-Carnitine Administration in Sheep Preceding an Oral Urea Drench

Introduction

Ammonia toxicity, a poorly understood metabolic disease, is known to cause derangements in intermediary metabolism (Chalupa, 1972; Spires and Clark, 1979; Visek, 1984). Specifically, as a result of elevated blood ammonia levels, ruminants experience hyperglycemia (Bartley et al., 1976; Emmanuel et al., 1982; Fernandez et al., 1988, 1990 a,b) because of increased glycogenolysis (Spires and Clark, 1979; Fernandez et al., 1990 a) and reduced glucose uptake resulting from insulin insufficiency (Sener et al., 1978; Fernandez et al., 1988, 1990 a,b). Subclinical ammonia toxicity, though not fatal, can result in the suboptimal performance of production livestock (Chalupa, 1972; Visek, 1984; Fernandez et al., 1988, 1997).

Currently, ammonia toxicity is untreatable and is almost 100% lethal in acute cases (Casteel and Cook, 1984; Visek, 1984; Haliburton and Morgan, 1989). The natural compound L-carnitine may provide protection against the deleterious effects of ammonia toxicity. In mice, L-carnitine has been shown to reduce ammonia-related deaths in response to administration of lethal amounts of ammonium acetate (Grisolia et al., 1984; Costell et al., 1987; O'Connor et al., 1987). Studies using Channel catfish and Chinook salmon have also shown that L-carnitine increases the tolerance to environmental ammonia (Burtle and Newton, 1991; Tremblay and Bradley, 1992).

The objectives of these experiments were to investigate the effects of intravenous administration of L-carnitine in sheep, and to determine its ability to ameliorate subclinical hyperammonemia experimentally induced using an oral urea drench.

Materials and Methods

Experiment 1. Six mature nonpregnant Suffolk ewes (avg BW 75 ± 3 kg) were used to investigate the effects of intravenous L-carnitine administration on selected plasma metabolites. Ewes were housed in indoor pens and individually fed 300 g/d of an alfalfa-based pellet ration twice daily (0800 and 1600 hr) plus bermudagrass hay to maintain BW (NRC, 1985). Ewes had ad libitum access to a trace mineral block and water. Ewes were allowed 21 d to adjust to the diet and handling. The experiment was conducted as a 3 x 3 Latin Square design. One week separated each experimental period to avoid carry-over effects. On experimental days, ewes were fasted overnight (16 hr) and were haltered, tethered, and fitted with sterile indwelling jugular vein catheters (Quik-Cath®, 14G 5.1 cm; Baxter Healthcare Corp., Deerfield, IL) and allowed 1 hr to rest. Treatments were: 0 (CONT), 6.36 (CAR 1) and 12.72 (CAR 2) mmol L-carnitine/kg $^{.75}$ BW. The L-carnitine (99+% Pure L-carnitine, Lot # 18.8.94; Lonza, Inc., Fair Lawn, NJ) was dissolved in deionized water as a 50% w/v solution. The L-carnitine solutions and the saline were adjusted to have similar pH (8.9 and 8.8, respectively). Blood was sampled at -15, 0, 15, 30, 60, 90, 120, 180, 240, 300 and 360 min relative to administration of the treatment. The treatment solutions were administered as intravenous bolus doses 15 min after the start of sampling. Blood (5 mL) was collected via the jugular catheters and immediately placed into vacutainer tubes containing either potassium oxalate as the anticoagulant plus

sodium fluoride (glycolytic inhibitor) or containing only the anticoagulant K_3EDTA (Monoject Blood Collection Tubes; Sherwood Medical, St. Louis, MO). Plasma was analyzed for glucose and NEFA, and plasma L-carnitine concentrations were determined in the 0, 60 and 240 min samples.

Experiment 2. Sixteen spring-born Suffolk ewe lambs (avg. BW 48 ± 2 kg) were used to investigate the effects of an intravenous administration of L-carnitine preceding experimentally-induced subclinical hyperammonemia. Lambs were fed once daily (0800) a pelleted total mixed ration formulated to promote growth and meet the recommended nutrient requirements (NRC, 1985). Bermudagrass hay and water were available ad libitum.

Body weights were recorded at 0730 after a 16 hr fast on days 0 and 9 of the trial. On Day 9, lambs were tethered, fitted with sterile indwelling jugular vein catheters (Quik-Cath®, 14G 5.1 cm) and allowed 1 hr to rest. The experiment was conducted as a randomized complete block design with a 2 x 2 factorial arrangement of treatments. The L-carnitine-saline solution (0 and 6.36 mmol/kg$^{.75}$ BW) was administered via the jugular vein catheter following the 30 min blood sample, and an oral urea load test (OULT; 0 and 300 mg/kg BW) was administered using a stomach tube following the 60 min blood sample. The L-carnitine and urea solutions were freshly prepared by dissolving L-carnitine or urea into deionized water as a 50% w/v solution. The L-carnitine solution and the saline were similar in pH (8.01 and 8.35, respectively).

Blood samples were collected via the jugular vein catheter into 4-mL tubes containing potassium oxalate and sodium fluoride (Monoject Blood Collection Tubes) at

0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, 240, 270, 300, 330, 360, 390, and 420 min. All plasma samples were analyzed for ammonia nitrogen, glucose, urea nitrogen, NEFA, and insulin, whereas plasma L-carnitine concentrations were determined in the 0, 60, 120, 180, 240, 300, 360, and 420 min samples. Ruminal fluid samples were collected via stomach tube immediately prior to the oral urea drench and 30 min after drenching. Rumen fluid samples were immediately placed in bottles containing 500 μL of saturated mercuric chloride as the preservative and analyzed for pH and ammonia nitrogen.

Sample Handling and Analytical Procedures. Blood samples were collected via the jugular vein catheters into 4-mL tubes containing potassium oxalate and sodium fluoride. Samples were centrifuged (4° C) at 1600 x g for 15 min and the plasma harvested and stored at -20° C until analyzed. Plasma metabolite concentrations were determined by spectrophotometric methods. Plasma was analyzed for ammonia nitrogen and urea nitrogen (Laborde et al., 1995), L-carnitine (Method No. 1-242-008; Boehringer Mannheim, Indianapolis, IN), glucose (Method No. 315; Sigma Chemical Co., St. Louis, MO), and NEFA (NEFA-C Kit, ACS-ACOD Method; Wako Chemicals USA, Inc., Richmond, VA). Samples were assayed in duplicate; and measurements resulting in a 5% error were reanalyzed. Plasma insulin concentrations were determined using a double-antibody radioimmunoassay procedure (Kitchalong et al., 1995). The mean intraassay and interassay CV for the insulin radioimmunoassay were 4.4 and 5.6%, respectively.

Ruminal fluid samples collected via stomach tube were analyzed for pH and ammonia nitrogen (Chaney and Marbach, 1962). Ruminal fluid free, nonionized ammonia nitrogen was calculated as described by Visek (1968):

$$\% \text{ Free, nonionized ammonia N} = 100 - (100 \div [1 + \text{antilog}(\text{pH} - \text{pK}'_a)])$$

using ruminal fluid pH and 9.02 as the pK'_a of the ammonium ion.

Statistical Analysis. Data from Exp. 1 were analyzed using the MIXED procedure of SAS (1992). Differences among least-squares treatment means were determined by ANOVA. Because of replicated measurements of the metabolites, data were analyzed as a Latin Square Design with repeated measures using the first-order autoregressive model (Gill, 1990). Data from Exp. 2 were analyzed as a randomized complete block design with a 2 x 2 factorial arrangement of treatments with a split plot in time using the GLM procedure of SAS (1992). The whole plot effects of urea level, carnitine level, and their interaction were tested using urea x carnitine as the error term. The subplot effects were tested using the residual error. Differences in treatment means were seperated using the Bonferroni method (Steel and Torie, 1980). Area under the response curve was calculated relative to basal levels using trapezoidal geometry for the time period 0 to 420 min after dosing. The data for area under the response curve was analyzed using the GLM procedure of SAS (1992).

Results

Experiment 1. The objective of experiment 1 was to investigate the effects of intravenous L-carnitine administration on selected plasma metabolites in sheep. Plasma L-carnitine concentrations were increased ($P < .05$) by treatment (51.9 vs 102.3, and 96.4 μmol/L in CONT, CAR 1, and CAR 2 groups, respectively; Figure 4.1). Post-dose plasma glucose (mean Pre-dose = 3.19 mmol/L; Figure 4.1) concentrations were elevated ($P < .05$) in the CAR 2 and CAR 1 treatment groups as compared with the CONT (3.71 and 3.56 vs 3.17 mmol/L). Plasma glucose peaked 170% above pre-dose at 15 min ($P < .001$) in the CAR 2 group and remained elevated until 90 min post-dose. Plasma glucose peaked 162% above pre-dose at 15 min ($P < .007$) in the CAR 1 group and remained elevated until 60 min post-dose. Area under the response curve (AUC) for glucose was greater ($P < .02$) in the CAR 2 (174.3 mM • min) as compared with CONT (-31.6 mM • min) and CAR 1 (44.3 mM • min; Table 4.1). Post-dose plasma NEFA concentrations (mean pre-dose = 155 μEq/L; Figure 4.1) were higher ($P < .05$) in the CAR 2 treatment as compared with the CONT and CAR 1 (234 vs 143 and 138 μEq/L). Plasma NEFA peaked 198% above pre-dose at 15 min ($P < .001$) in the CAR 2 group as compared to the CAR 1 and CONT groups (477 vs 161 and 139 μEq/L; respectively). The AUC for NEFA was not affected ($P > .10$) at any level of L-carnitine administration (Table 4.1).

Experiment 2. The objective of experiment 2 was to determine the effects of intravenous L-carnitine administration during experimentally induced subclinical hyperammonemia in sheep. Plasma L-carnitine was increased ($P < .0001$) by 30 min after L-carnitine administration in both the Carnitine and Carnitine + Urea treatment groups,

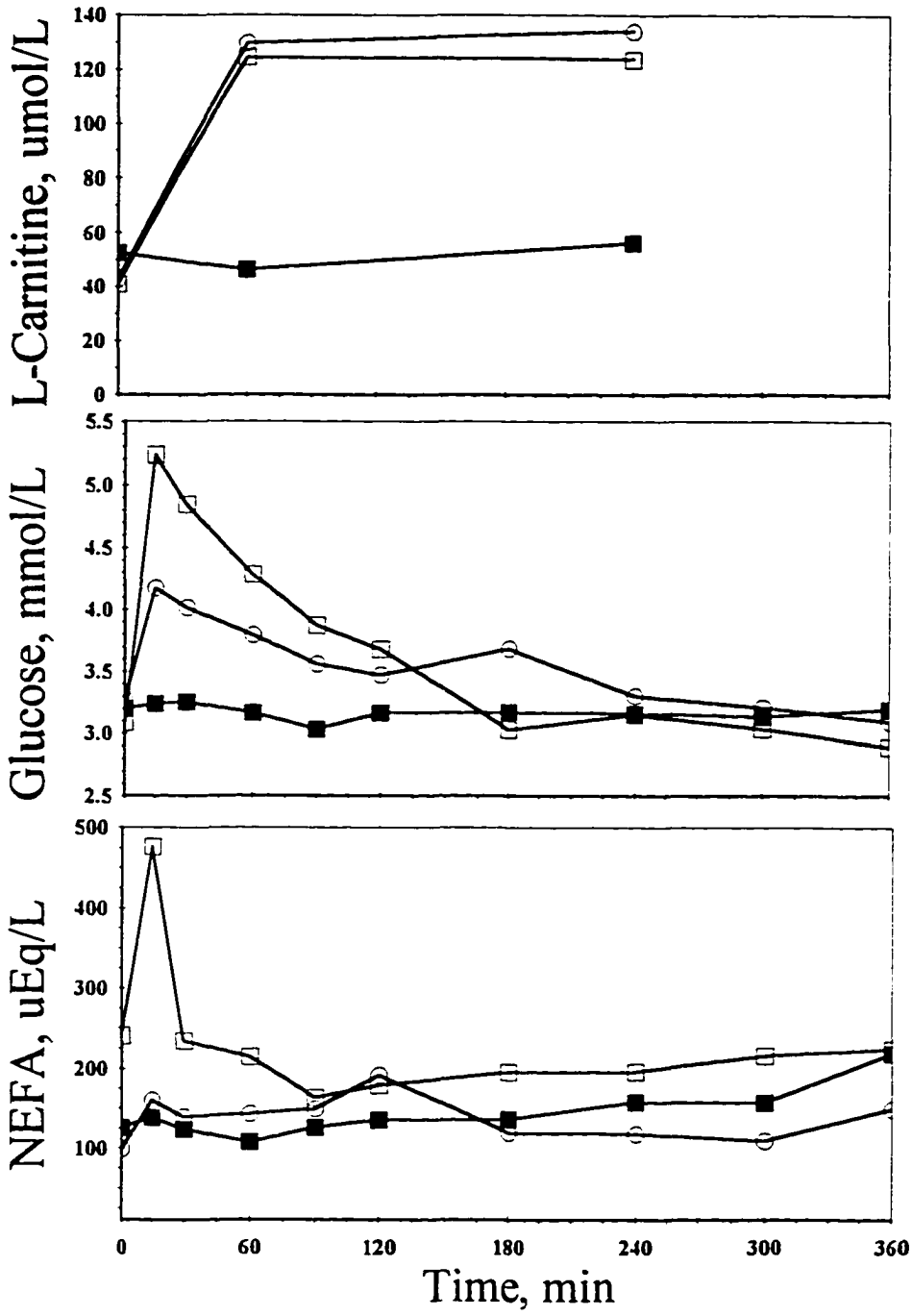

Figure 4.1. Effect of intravenous L-carnitine administration in sheep on plasma concentrations of L-carnitine, glucose, and NEFA (Exp. 1). Dose levels of L-carnitine were Cont = 0 mmol/kg$^{.75}$ BW (■), CAR 1 = 6.36 mmol/kg$^{.75}$ BW(□), and CAR 2 = 12.72 mmol/kg$^{.75}$ BW (○). Values represent the least square means of six ewes. The pooled SE from the analysis of variance for L-carnitine, glucose, and NEFA concentrations were 19.9 μmol/L, 0.2 mmol/L, and 42.9 μEq/L, respectively.

Table 4.1. Effect of intravenous administration of L-carnitine on plasma glucose and nonesterified fatty acid area under the response curve (Exp. 1)[a]

	Level of L-carnitine administration[b]			
Plasma criteria	CONT	CAR 1	CAR 2	SEM
Glucose, Min • mM[c]	- 31.6[x]	44.3[x]	174.3[y]	43.2
NEFA, Min • mEq/L	2.5	14.9	-1.6	24.1

[a] Values represent least square means of six ewes.
[b] Dose levels were CONT = 0 mmol/kg$^{.75}$ BW, CAR 1 = 6.36 mmol/kg$^{.75}$ BW, and CAR 2 = 12.72 mmol/kg $^{.75}$ BW.
[c] Overall treatment effect, $P < .02$.
[xy] Within a row, means lacking a common superscript letter differ, $P < .05$.

and remained elevated during the collection period (Figure 4.2). There were no treatment differences ($P > .10$) in ruminal fluid pH (overall mean 7.03 ± 0.06) (Figure 4.3). Ruminal fluid ammonia nitrogen (overall mean 15.2 ± 2.03 mmol/L) increased ($P < .001$) in the Urea and Carnitine + Urea groups 30 min after the administration of the OULT (Figure 4.3). Rumen fluid nonionized ammonia nitrogen was highest ($P < .01$) in the Urea and Carnitine + Urea groups 30 min after the OULT (Figure 4.3). Plasma ammonia nitrogen was highest ($P < .0001$) in the Urea treatment group compared to the Control, Carnitine, and Carnitine + Urea treatment groups (148 vs 95, 101, and 108 μmol/L, respectively) (Figure 4.4). Plasma urea nitrogen (overall mean 15.3 ± 0.17 mmol/L) exhibited a time x treatment interaction ($P < .0001$) with the Urea group showing an increase in concentration over the collection period while urea nitrogen levels in the other treatment groups remained relatively constant (Figure 4.4). Plasma glucose (overall mean 3.52 ± 0.03 mmol/L) was not affected by treatment ($P > .10$) (Figure 4.4); however, after administration of carnitine and again after the administration of urea, there was a transient increase in glucose concentrations which quickly returned to normal levels. There were no treatment effects ($P > .10$) on either plasma insulin (24.3 ± 1.2 μU/mL; not shown) or NEFA concentrations (108.1 ± 17.4 μEq/L; not shown). The AUC for glucose was affected by treatment ($P < .04$) with the Urea group producing the most negative AUC compared to the Control, Carnitine, and Carnitine + Urea treatment groups (Table 4.2). The AUC for plasma ammonia nitrogen, urea nitrogen, NEFA, and insulin were not affected ($P > .10$) by treatment (Table 4.2).

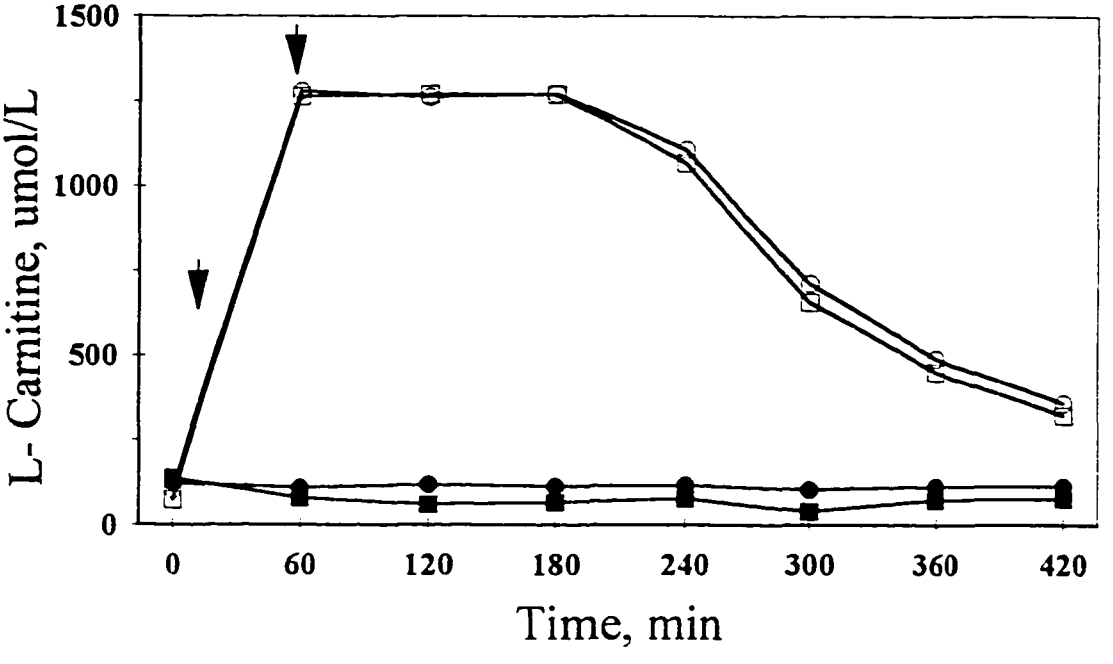

Figure 4.2. Effect of intravenous L-carnitine administration and oral urea load test on plasma L-carnitine concentration (Exp. 2). Treatments were: Control = saline (■); Carnitine = 6.36 mmol L-carnitine/kg$^{.75}$ BW (○); Urea = 300 mg urea/kg BW (●); and Carnitine + Urea = 6.36 mmol L-carnitine/kg$^{.75}$ BW + 300 mg urea/kg BW (□). The pooled SE was 16.8 μmol/L.

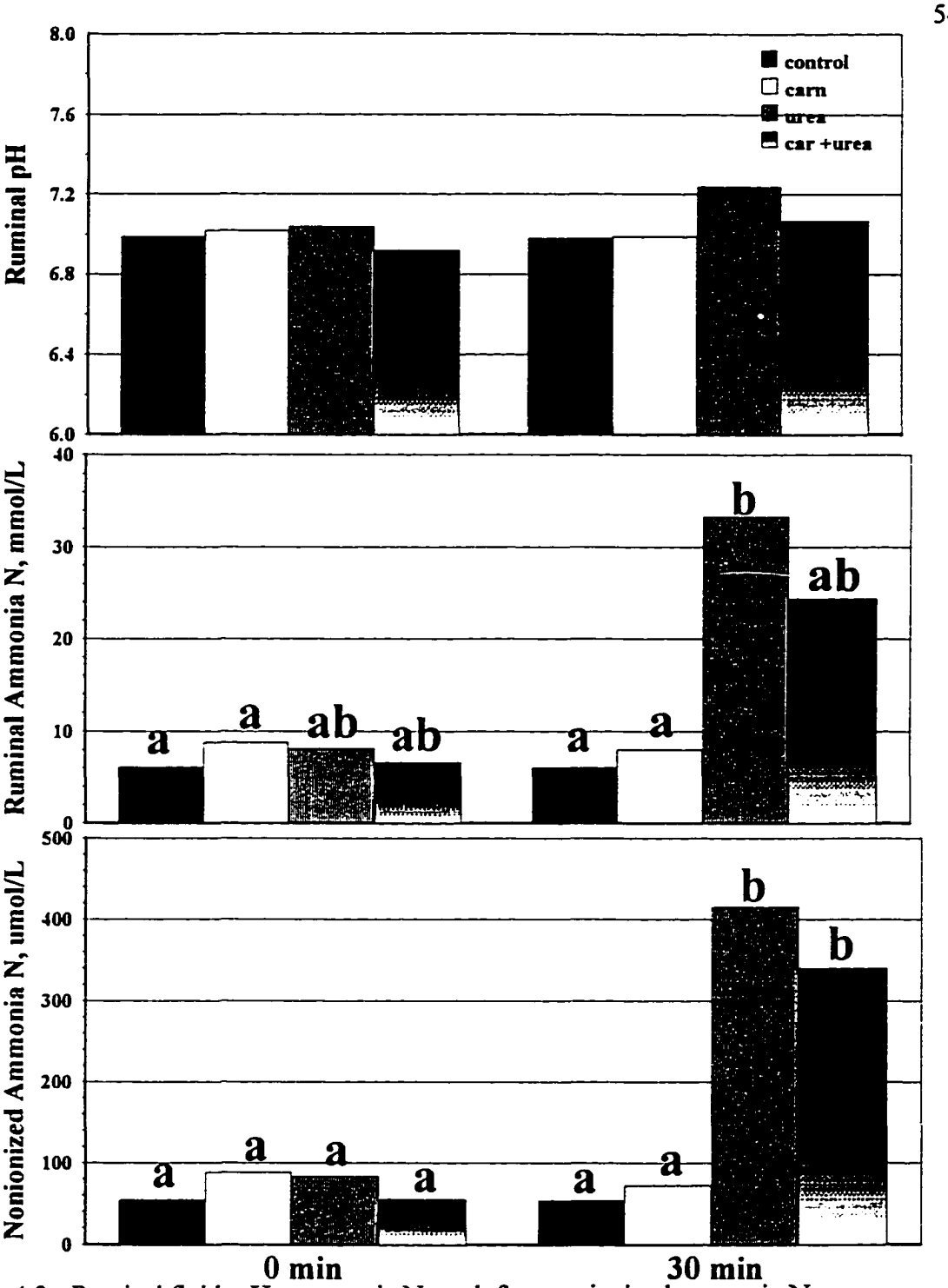

Figure 4.3. Ruminal fluid pH, ammonia N, and free nonionized ammonia N concentrations in sheep following intravenous L-carnitine administration and oral urea load test (Exp. 2). Treatments were: Control = saline; Carnitine = 6.36 mmol L-carnitine/kg$^{.75}$ BW ; Urea = 300 mg urea/kg BW; and Carnitine + Urea = 6.36 mmol L-carnitine/kg$^{.75}$ BW + 300 mg urea/kg BW. The pooled SE for pH, ammonia N, and free nonionized ammonia N were 0.05, 1.4 mmol/L, and 38.3 µmol/L, respectively.

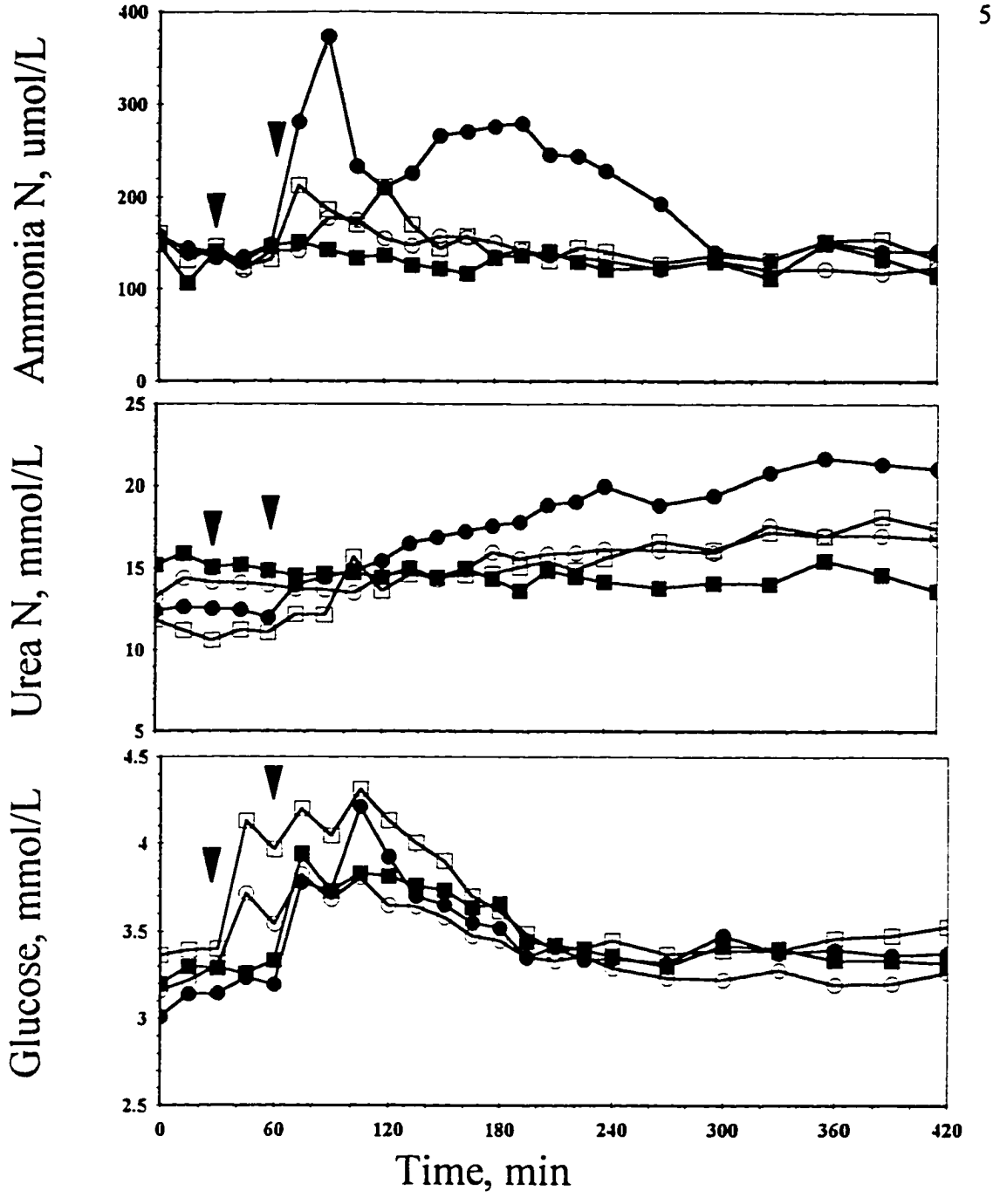

Figure 4.4. Changes in plasma ammonia N, urea N, and glucose concentrations in sheep following intravenous L-carnitine administration and oral urea load test (Exp. 2). Arrows denote when treatments were administered. The first arrow denotes the administration of the four treatments and the second represents the start of the oral urea load test. Treatments were: Control = saline (■); Carnitine = 6.36 mmol L-carnitine/$kg^{.75}$ BW (○); Urea = 300 mg urea/kg BW (●); and Carnitine + Urea = 6.36 mmol L-carnitine/$kg^{.75}$ BW + 300 mg urea/kg BW (□). The pooled SE for ammonia N, urea N, and glucose were 25.2 μmol/L, 0.8 mmol/L, and 0.1 mmol/L, respectively.

Table 4.2. Effect of intravenous administration of L-carnitine and oral urea load test on area under the response curve of plasma metabolites and insulin (Exp. 2)[a]

Plasma criteria	Treatments[b]				SEM
	Control	Carnitine	Urea	Carnitine + Urea	
Ammonia N, min • μmol/L	6437.4	-19237.5	-489.2	11720.6	4748.8
Urea N, min • mM	149.8	668.6	2038.6	-2277.2	748.3
Glucose, min • mM	141.1[x]	-85.4[xy]	-812.7[y]	476.5[x]	116.8
NEFA, min • mEq/L	-46.8	-44.1	-22.4	48.7	25.0
Insulin, min • μU/mL	-991.2	2217.3	4240.2	-3701.4	1077.4

[a] Values represent the least square means of four sheep within each treatment.
[b] Treatment levels were Control = saline; Carnitine = 6.36 mmol L-carnitine/$kg^{.75}$; Urea = 300 mg urea/kg BW; Carnitine + Urea = 6.36 mmol L-carnitine/$kg^{.75}$ and 300 mg urea/kg BW.
[xy] Within a row, means lacking a common superscript letter differ, $P < .05$.

Discussion

Hyperammonemia, also known as ammonia toxicity, can be characterized by elevated levels of circulating ammonia (Visek, 1968,1984; Chalupa, 1972; Casteel and Cook, 1984). The elevated levels of ammonia in circulation are associated with the quick absorption of the nonionized form of ammonia (NH_3 vs NH_4^+) from the rumen under conditions of elevated pH and excess ammonia (Bartley et al., 1981; Huntington, 1986; Haliburton and Morgan, 1989). Acute cases of hyperammonemia are lethal; and the only effective method of treating acute cases is total rumen evacuation (Bartley et al., 1976, 1981; Casteel and Cook, 1984; Haliburton and Morgan, 1989). In a production setting, this method is not practical. L-carnitine may provide a practical means of preventing hyperammonemia in ruminants.

The administration of L-carnitine in mice has been shown to provide protection against the effects of known lethal levels of ammonium acetate (Grisolia et al., 1984; Matsuoka et al., 1991; Matsuoka and Igisu, 1993) and against the toxicity of ammonia induced by sodium benzoate (O'Connor et al., 1987; Michalak and Qureshi, 1990; Ratnakumari et al., 1993). However, other studies have not shown the protective effect of L-carnitine against toxicity (Deshmukh and Rusk, 1988; Deshmukh et al., 1990). Since the mechanism of ammonia toxicity is poorly understood, the mechanism by which L-carnitine may afford protection is also poorly understood.One possibility is that L-carnitine may be stabilizing liver mitochondrial membranes therefore improving the structural and metabolic integrity of the mitochondria (Di Lisa et al., 1985; Bellei et al., 1989). It has been suggested that the adverse effects due to ammonia may be attributed to

derangements (i.e. decrease) in the intramitochondrial NADH/NAD^+ ratio (Feldman, 1971; Spires and Clark, 1975; Grisolia et al., 1984). Since L-carnitine enhances the rate of fatty acid β-oxidation in the hepatocytes, it is possible that an increase in β-oxidation could increase the intramitochondrial NADH concentrations (Giesecke, 1983; Madsen, 1983) therefore, alleviating the adverse affects attributed to ammonia. In contrast, Kloiber et al. (1988) suggested that the effectiveness of L-carnitine may be due to osmoregulation.

There have been few studies measuring ruminal characteristics after L-carnitine administration. Ruminal fluid pH was not affected by intravenous L-carnitine administration in Exp. 2 which agrees with LaCount et al. (1995, 1996a). As expected, ruminal fluid ammonia nitrogen was not affected by intravenous L-carnitine administration; however, administration of the OULT resulted in the expected increase in ruminal fluid ammonia nitrogen (Visek, 1968; Chalupa, 1972; Spires and Clark, 1979). The increase in rumen ammonia nitrogen after an oral urea dosage has also been observed in cattle (Spires and Clark, 1979), sheep (Emmanuel and Edjtehadi, 1981; Emmanuel et al., 1982), and goats (Fernandez et al., 1990 b). In the rumen, urea is rapidly hydrolyzed to ammonia which is absorbed through the rumen epithelium (NRC, 1976; Church, 1988). Total rumen ammonia concentration is composed of NH_4^+ plus NH_3 with the majority being in the ionized form (NH_4^+) (Visek, 1968; Chalupa, 1972). The percent of ammonia as NH_3 was calculated as previously described, (Visek, 1968) and represents the portion of free, nonionized ammonia which can readily diffuse across the rumen epithelium into the portal and systemic circulation (Visek, 1968; Chalupa, 1972; Huntington, 1986). In Exp. 2 the free, nonionized portion of rumen ammonia nitrogen was highest in the ewes

receiving the OULT. This increase in ruminal free ammonia was reflected in a concomitant increase in plasma ammonia nitrogen concentrations. However, the animals which were pre-treated with L-carnitine had significantly lower plasma ammonia nitrogen levels compared with the animals receiving urea but no L-carnitine.

In Exp. 2, sheep treated with L-carnitine prior to the OULT had significantly lower levels of plasma ammonia nitrogen when compared with the sheep that did not receive the L-carnitine. These findings agree with Igisu et al. (1995) who found that L-carnitine lowered the concentration of ammonia in both blood and in the brain during ammonia intoxication. In both Exp. 1 and 2, L-carnitine was administered intravenous and increased in the blood of sheep treated with L-carnitine and remained elevated for the duration of the sampling time (6 hr). Studies in cattle (LaCount et al., 1995; LaCount et al., 1996 a,b) and mice (Costell et al., 1987) have shown that when L-carnitine is fed or injected into the rumen, abomasum, or peritoneum, plasma levels of L-carnitine increase linearly. In mice, the route of administration may influence the effectiveness of L-carnitine since O'Connor et al. (1986) found that intraperitoneal injections offered the most protection followed by intravenous and intramuscular administrations, with subcutaneous administration of L-carnitine offering the least amount of protection against acute ammonia intoxication. O'Connor et al. (1986) suggested that the differences in effectiveness due to route of administration may be due to the rate of absorption or distribution of the absorbed carnitine. In ruminants, the effectiveness of L-carnitine may be influenced by the microbial population of the rumen. LaCount et al. (1996a) found that degredation of L-carnitine was greater in ruminal fluid from cows that had adapted to

dietary carnitine supplementation for 2 wk. In Exp. 1, plasma glucose levels were increased in the CAR 1 and CAR 2 treatment groups. Erfle et al. (1971) also found an increase in plasma glucose levels in spontaneously ketotic cows that received an intravenous infusion of 80 g of L-carnitine. In Exp. 2, plasma glucose showed only a transient increase after intravenous L-carnitine administration, and then quickly returned to baseline concentrations. LaCount et al. (1995, 1996 a,b) reported no effect of L-carnitine on plasma glucose concentrations in dairy cattle regardless of whether L-carnitine was fed or infused into the rumen or abomasum. The transient rise in plasma glucose observed in the present experiment could be due to the concentration of L-carnitine used or the duration of the treatment (bolus administration vs. infusion). In Exp. 2, plasma glucose levels also increased transiently after the OULT was administered but quickly returned to pre-OULT levels. Spires and Clark (1979) and Symonds et al. (1981) observed that plasma glucose levels increased in cattle administered a urea solution directly into the rumen. The same increase in plasma glucose was seen in sheep (Emmanuel and Edjtehadi, 1981) and dairy goats given a urea solution orally (Fernandez et al., 1990 b). It has been reported that hyperammonemia results in an impaired insulin response to hyperglycemia in isolated rat islets (Sener et al., 1978) and steers (Fernandez et al., 1988). Plasma insulin was not affected by treatment in Exp. 2.

Exp. 1 showed an increase in plasma NEFA in the CAR 2 treatment group compared to the other groups. However, in Exp. 2, plasma NEFA was not affected by treatment. Several studies (LaCount et al., 1995, 1996 a,b) have reported that L-carnitine did not affect plasma NEFA. Nevertheless, Erfle et al. (1971) reported a decrease in the

concentration of plasma NEFA in feed-restricted cattle when L-carnitine was infused intravenously. The NEFA response in Exp. 1 could be due to the level (12.72 mmol/kg $^{.75}$ BW) at which it was administered; however, the AUC was not affected by any of the treatments.

Another possible hypothesis of L-carnitine's mode of action in preventing acute ammonia intoxication is through the enhanced incorporation of ammonia into urea (O'Connor et al., 1987). O'Connor et al. (1987) observed that mice treated with L-carnitine (16 mmol/kg BW) experienced a continuous rise in blood urea nitrogen until a plateau was reached 1 h after injection, whereas mice not treated with L-carnitine experienced a rise in blood urea nitrogen, but died within 15 min of ammonium acetate administration. Plasma urea nitrogen in Exp. 2 was not affected by treatment; however, there was a treatment x time interaction in which the plasma urea nitrogen levels in the ewes not treated with L-carnitine experienced a continuous rise in plasma urea nitrogen throughout the sampling period while the other treatment groups remained relatively constant. The continuous increase in urea nitrogen concentrations could be a reflection of the plasma ammonia nitrogen concentrations which were highest in the ewes not given L-carnitine (Exp. 2). Plasma ammonia nitrogen would be converted to urea nitrogen via the urea cycle (Visek, 1984). Because the plasma ammonia nitrogen concentrations for the Control, Carnitine, and Carnitine + Urea groups were significantly lower when compared to the Urea group, it would follow that the urea nitrogen concentrations would also be lower.

In both Exp. 1 and 2, intravenous administration of L-carnitine resulted in either no changes or transient changes in key energy metabolites. In addition, L-carnitine administration did not alter ruminal fluid pH, ammonia nitrogen, or nonionized ammonia nitrogen levels. Despite these results, plasma ammonia nitrogen concentrations were lower in animals receiving L-carnitine prior to oral urea. Further research is warranted to elucidate a possible mechanism by which L-carnitine is providing protection against hyperammonemia.

Implications

Intravenous L-carnitine significantly lowered plasma ammonia nitrogen levels in ewes given an oral urea challenge even though the concentration of rumen free, nonionized ammonia nitrogen were similar to the ewes treated only with the urea solution. This suggests that L-carnitine administration may prevent subclinical hyperammonemia in ruminants.

Chapter 5

Influence of Dietary Carnitine in Growing Sheep Fed Rations Containing Nonprotein Nitrogen

Introduction

Carnitine, a naturally occuring quaternary amine compound, is necessary for the transportation of long chain fatty acids across the inner mitochondrial membrane (Stryer, 1988). Although carnitine is not considered to be an essential nutrient for mammals, studies in swine (Owen et al., 1996; Musser et al., 1997) and cattle (Staples et al., 1975; LaCount et al., 1995; LaCount et al., 1996a,b) have shown that the addition of L-carnitine to diets may prove beneficial for production. Weeden et al. (1990) found that supplemental L-carnitine fed from day 14 to 35 postweaning improved the gain:feed ratio in pigs. Finishing pigs fed added L-carnitine had better feed efficiency and had less average backfat thickness compared with pigs fed no L-carnitine (Newton and Haydon, 1989; Weeden et al., 1991). Additionally, Owen et al. (1993, 1994b, 1996) reported that lipid accretion was reduced in pigs fed 750 to 1,000 ppm L-carnitine. Higher average daily gains and better efficiency were reported in growing-finishing swine fed 50 ppm L-carnitine (Owen et al., 1994a; Smith et al., 1994). L-carnitine's potential for improving carcass characteristics in pigs was shown by Owen et al. (1993) who reported an increase in longissimus muscle area and a reduction in daily lipid accretion rate and a reduction in backfat thickness in growing-finishing pigs. Supplementing sows with L-carnitine

shortened the time between weaning and sows' first estrous, reduced piglet mortality rate, and increased litter weaning weights (Fremaut et al., 1993; Harmeyer 1993; Musser et al., 1997).

Responses in ruminants to L-carnitine administration have been variable. Supplemental L-carnitine, administered directly either into the rumen or abomasum, was effective in increasing plasma, liver, and milk concentrations of L-carnitine; however, L-carnitine had little effect on milk yield and composition (LaCount et al., 1995). Subcutaneous injections of L-carnitine had no effect on milk or component yields (Staples et al., 1975). Yavuz et al. (1997) reported that supplemental L-carnitine reduced plasma urea nitrogen concentrations in Holstein calves fed diets containing 50% broiler litter. Supplemental L-carnitine also improved growth in grazing beef calves fed liquid supplements containing urea and was associated with reduced ruminal ammonia nitrogen levels (White et al., 1997). A study using heifers (Hill et al., 1995) showed that dietary L-carnitine reduced marbling score, but little to no consistent effects have been found in steers (Hill et al., 1994). L-carnitine has also been shown to protect mice against known lethal amounts of ammonium acetate (Grisolia et al., 1984; Costell et al., 1987; O'Connor et al., 1987). Studies using Channel catfish have also shown that L-carnitine increases the tolerance to environmental ammonia (Burtle and Newton, 1991). The objective of this experiment was to determine the influence of supplemental L-carnitine on growth and metabolic criteria of growing lambs fed a diet high in NPN (comprising 50% of total dietary N).

Materials and Methods

Animal Care. Twenty-three Spring-born (Born February - March 1997) Suffolk lambs (13 wethers and 10 ewe lambs) were used in a feeding trial (June 1997- August 1997) to investigate the effects of supplemental L-carnitine on growth and metabolic criteria of growing lambs fed a diet high in NPN (comprising 50% of total dietary N). The lambs, obtained from the LAES Sheep Unit, were stratified by weight and blocked by sex and randomly assigned to pens. Prior to initiation of the experiment, the lambs were dewormed, and feet properly trimmed. Lambs were housed in individual indoor pens with a concrete floor. Lambs were individually fed and slowly adapted (over 21 days) to the diets containing NPN as urea and supplemental 50 % L-carnitine (Carniking Lot # 101031; Lonza, Inc., FairLawn, NJ) was added to the appropriate diets on Day 1 of the collection period. This was done to insure that any adaptation by the rumen microorganisms to L-carnitine would occur during the collection period. During the 50 day collection period, lambs were individually fed (0730) at a level to insure 15 to 20 % orts, and had ad libitum access to the diets and water. Individual intakes were recorded daily, and the orts were removed prior to feeding. The concentrate-based experimental diet was formulated to meet the recommended nutrient requirements of growing lambs (NRC, 1985; Table 5.1). Treatments included two levels of dietary NPN as urea (0 and 50% of total dietary N) and supplemented with two levels of 50 % L-carnitine (0 or 499 ppm of feed).

Table 5.1. Composition of experimental diets.

	Diets[a]			
Ingredients	Control	NPN	Carn	NPN+Carn
Feed Composition (as fed basis)				
Corn	30.63	52.32	29.63	51.32
Cottonseed hulls	43.74	37.50	43.74	37.50
Soybean meal (49%CP)	18.60	0.14	18.60	0.14
Urea	--	2.68	--	2.68
Oystershell flour	0.59	0.48	0.59	0.48
Trace Mineral salt[b]	0.32	0.33	0.32	0.33
Vitamin premix[c]	0.10	0.10	0.10	0.10
Monocalcium phosphate	1.01	1.45	1.01	1.45
Carnitine premix[d]	--	--	1.00	1.00
Bovatec[e]	5.00	5.00	5.00	5.00
Nutrient Compositon (% of DM)				
DM	94.96	95.58	94.61	95.43
CP	12.09	13.57	12.27	13.12

[a] Experimental diets were Control = urea contributing 0% of the total dietary N + 0 g L-Carnitine; NPN = urea contributing 50% of the total dietary N + 0 g of L-Carnitine; Carn = urea contributing 0% of the total dietary N + 499 ppm of L-Carnitine; NPN + Carn = urea contributing 50% of the total dietary N + 499 ppm of L-Carnitine

[b] Trace mineral salt provided the following per kilogram of diet: sodium chloride, 4.4 g; manganese, 14 mg; iron, 8.5 mg; copper, 2.3 mg, iodine, .23 mg, cobalt, .24 mg.

[c] Provided the following per kilogram of diet: riboflavin, 2.6 mg; pantothenic acid, 10 mg; niacin, 18 mg; vitamin B12, 12 μg; biotin, 88 μg; choline, 176 mg; menadione, 1.7 mg; folic acid, .7 mg; thiamine, .9 mg; pyridoxine, .9 mg; ascorbic acid, 22 μg; vitamin A, 2205 IU; vitamin D, 661 IU; vitamin E, 18 IU; .

[d] Carnitine premix will consist of 499 ppm of 50 % L-carnitine

[e] Bovatec (15% mixture) was added at a rate of 136 g / 907 kg of diet.

Daily Sampling. On Days 1, 8, 29, and 50 of the collection period, following a 16 hr fast, lambs were weighed and blood was collected via jugular venipuncture into 7-mL vacutainer tubes containing potassium oxalate and sodium fluoride (Monoject Blood Collection Tubes; Sherwood Medical, St. Louis, MO) at 0, 1, and 3 hr post-feeding. Ruminal fluid samples (15 mL) were collected via stomach tube at 1 hr post-feeding and immediately placed in bottles containing 500 μL of saturated mercuric chloride as the preservative. Blood samples were placed into an ice bath, transported to the laboratory, centrifuged (4° C) at 1600 x g for 15 min, and then the plasma was harvested and stored at -20°C until analyzed.

Oral Urea Load Test. An oral urea load test was conducted on days 10 (OULT 1) and 50 (OULT 2) of the collection period. Following a 16-hr fast, lambs were fitted with a sterile indwelling jugular vein catheter (Quik-Cath®, 14G 5.1 cm; Baxter Healthcare Corp., Deerfield, IL), tethered, and allowed 1 hr to rest. A freshly prepared urea solution was administered as an oral urea drench (0.835 g/kg$^{.75}$ BW prepared with dH_2O as a 25% w/v solution) using a stomach tube at the 30 min blood sample. Blood samples were collected via the jugular vein catheter into 4-mL tubes containing potassium oxalate and sodium fluoride (Monoject Blood Collection Tubes) at 0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, 240, 270, 300, 330, 360, 390, and 420 min. Ruminal fluid samples were collected via stomach tube immediately prior to the oral urea drench (at the 30 min sample) and immediately placed in bottles containing saturated mercuric chloride as the preservative.

Chemical Analyses. Plasma from the samples collected on days 0, 8, 29, and 50 at 0, 1, and 3 hr post-feeding were analyzed for plasma ammonia nitrogen (Laborde et al., 1995), urea nitrogen (Laborde et al., 1995), glucose (Method No. 315; Sigma Chemical Co., St. Louis, MO), NEFA (NEFA-C Kit, ACS-ACOD Method; Wako Chemicals USA, Inc., Richmond, VA), and albumin (Laborde et al., 1995). Plasma insulin concentrations were determined using a double-antibody radioimmunoassay procedure (Kitchalong et al., 1995). The mean interassay CV for the insulin radioimmunoassay was 14%; additionally, plasma from the 0 hr of each day were analyzed for total carnitine (Minkler and Hoppel, 1993), triiodothyronine (T_3; ICN Kit # 07-292102, ICN Biomedicals, Costa Mesa, CA), thyroxine (T_4; ICN Kit # 07-290102, ICN Biomedicals), and cortisol (ICN Kit # 07-221102, ICN Biomedicals). The mean intraassay CV for triiodothyronine, thyroxine, and cortisol were 5.4%, 10%, and 2%, respectively. Samples were assayed in duplicate; and measurements resulting in a 7% error were reanalyzed. Ruminal fluid samples collected via stomach tube were analyzed for ruminal ammonia-N (Fernandez et al., 1997), and pH.

All plasma samples from the OULT 1 and OULT 2 were analyzed for plasma ammonia nitrogen, urea nitrogen, glucose, and insulin concentrations. The mean interassay CV for the insulin radioimmunoassay was 14% for the OULT 1 and 13% for the OULT 2. Additionally, plasma samples collected at the 0 min were analyzed for albumin, ammonia nitrogen, glucose, NEFA, urea nitrogen, and insulin and ruminal fluid samples were analyzed for pH and ruminal ammonia nitrogen. Samples were assayed in duplicate and measurements resulting in a 7% error were reanalyzed.

Statistical Analyses. Production data, daily plasma samples, and both OULT were analyzed using the MIXED procedure of SAS (1992). Data were analyzed as a randomized complete block design with sex as block with individual lamb as the experimental unit. The treatment structure was a 2 x 2 factorial arrangement with the two factors including two levels of urea and two levels of L-carnitine. The main effects tested were gender, level of L-carnitine, level of urea, time, and their interaction. Differences among least-square treatment means were separated using the Bonferroni method (Steel and Torrie, 1980).

Results

Intake and weight response. There were no differences in total feed intake on an "as fed" basis due to treatment ($P > .10$; Table 5.2). Although total feed intake was not different between treatments, intakes tended to be higher in lambs on Carn + NPN diet ($P = .11$) from day 1 to 7. Intake was increased over the experimental period ($P < .0001$). There was no differences in weight due to treatment ($P > .10$; Table 5.2). However, average daily gain (ADG) and gain:feed ratio were lower ($P < .0001$) in lambs on the NPN diets (Table 5.2). Body weight did increase over time ($P < .0001$) with all animals gaining weight throughout the collection period. Body weights exhibited a NPN x Day interaction ($P < .0001$; Figure 5.1) in which lambs consuming diets without NPN gained more weight when compared to the lambs consuming diets with NPN.

Daily Plasma and Ruminal Fluid Response. Lambs consuming the NPN diets had 13% higher ruminal fluid pH ($P < .0001$) compared to lambs consuming the non-NPN

Table 5.2. Effect of NPN and L-carnitine on initial BW, final BW, and ADG.

	Diets[a]				
Item	Control	NPN	Carn	NPN+Carn	SEM
Initial BW, kg	26.8	28.6	26.8	27.7	2.2
Final BW, kg	39.3	34.0	38.5	36.5	0.26
ADG, g/d	221.0 [x]	124.5 [y]	207.0 [x]	118.3 [y]	8.35

[a] Experimental diets were Control = urea contributing 0% of the total dietary N + 0 ppm L-Carnitine; NPN = urea contributing 50% of the total dietary N + 0 ppm of L-Carnitine; Carn = urea contributing 0% of the total dietary N + 499 ppm of L-Carnitine; NPN + Carn = urea contributing 50% of the total dietary N + 499 ppm of L-Carnitine.

[xy] Within a row, means lacking a common superscript letter differ, $P < .001$.

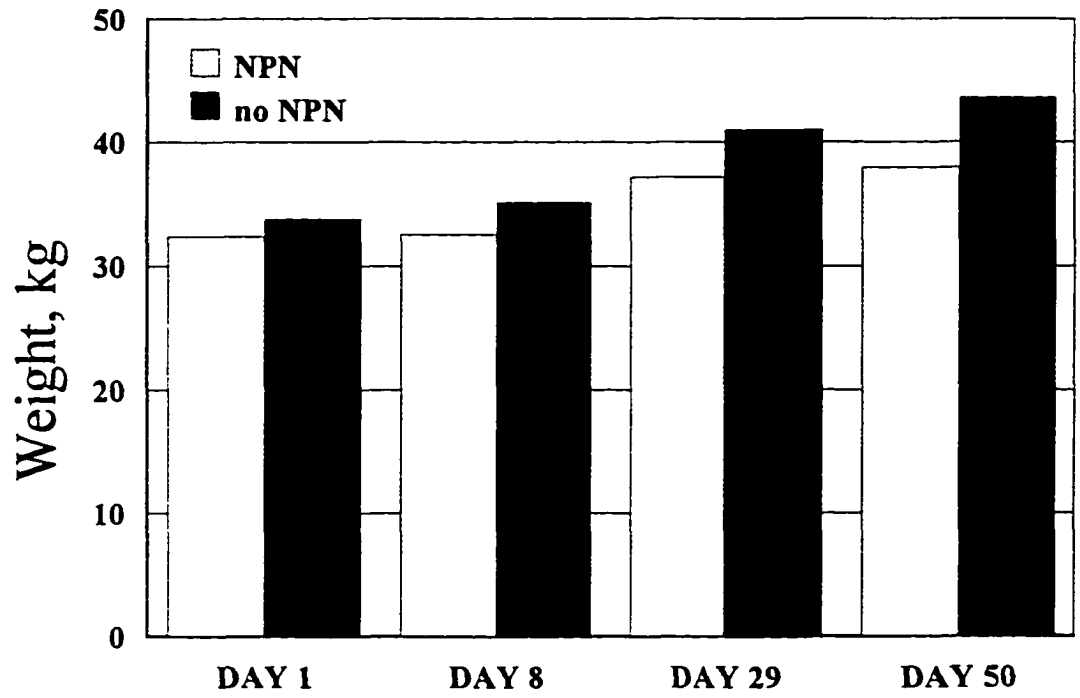

Figure 5.1. Effect of NPN and day on lamb weights. Experimental diets were NPN = urea contributing 50% of the total dietary N; no NPN = urea contributing 0% of the total dietary N. The pooled SE was 0.26 kg.

diets (Figure 5.2A). Lambs on the NPN diets had 72% higher ($P < .0001$) ruminal ammonia nitrogen compared with lambs not on the NPN diets (Figure 5.2B). Plasma total carnitine was 75% higher ($P < .002$) in the lambs fed L-carnitine compared with the lambs not fed L-carnitine (Figure 5.3A). Lambs fed the Carn diets had higher levels of plasma total carnitine at Day 8, 29, and 50 compared with lambs not fed the Carn diets (Carn x Day, $P < .04$; Figure 5.3B). Plasma ammonia nitrogen was 258% higher ($P < .0001$) in lambs fed the NPN diets compared with the lambs without NPN (Figure 5.4). Plasma ammonia nitrogen levels increased 1 hr post-feeding in lambs on the NPN diets but decreased in lambs not fed NPN (NPN x Hr, $P < .0001$; Table 5.3). There was no effect of Carn ($P > .10$) nor was there a NPN x Carn interaction ($P > .10$) for plasma ammonia nitrogen. Plasma glucose concentrations were 4% higher ($P < .05$) in the lambs on the Carn diets compared with the lambs not receiving Carn (Figure 5.4C). Lambs fed Carn had higher plasma glucose levels at Day 50 compared with lambs not fed Carn (Carn x Day, $P < .06$); additionally, lambs fed NPN had lower plasma glucose at 3 hr post-feeding compared with lambs not fed NPN (NPN x Hr, $P < .0001$; Table 5.3). Plasma urea nitrogen was 20% lower ($P < .003$) in the lambs on the NPN diets compared with lambs without NPN (Figure 5.4B). Plasma urea nitrogen levels increased at 1 and 3 hr post-feeding for the NPN diets while lambs not fed NPN exhibited a decrease in urea nitrogen (NPN x Hr, $P < .0001$; Table 5.3). Lambs on the NPN diets exhibited an increase in plasma NEFA levels 1 hr post-feeding but lambs fed the non-NPN diets showed a decrease during the same time period (NPN x Hr, $P < .0001$; Table 5.3). Plasma NEFA values were lower for the NPN treatment by Day 29 but then increased by Day 50. Lambs in the NPN + Carn treatment had lower NEFA levels by Day 8, but then increased

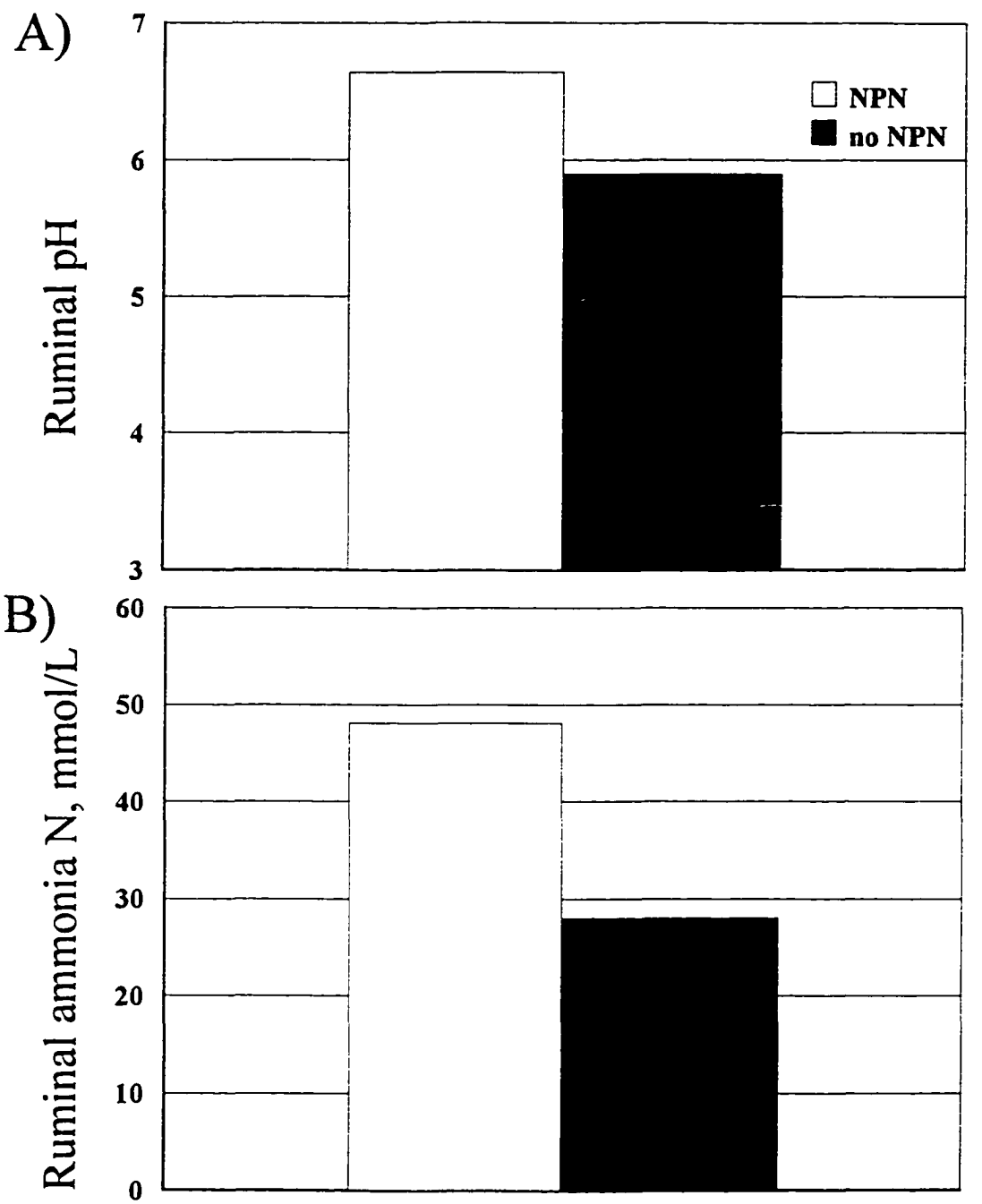

Figure 5.2. Effect of NPN on A) ruminal fluid pH and B) ruminal fluid ammonia N. Experimental diets were NPN = urea contributing 50% of the total dietary N; no NPN = urea contributing 0% of the total dietary N. The pooled SE was 0.01 and 18.6 mmol/L for pH and ammonia N, respectively.

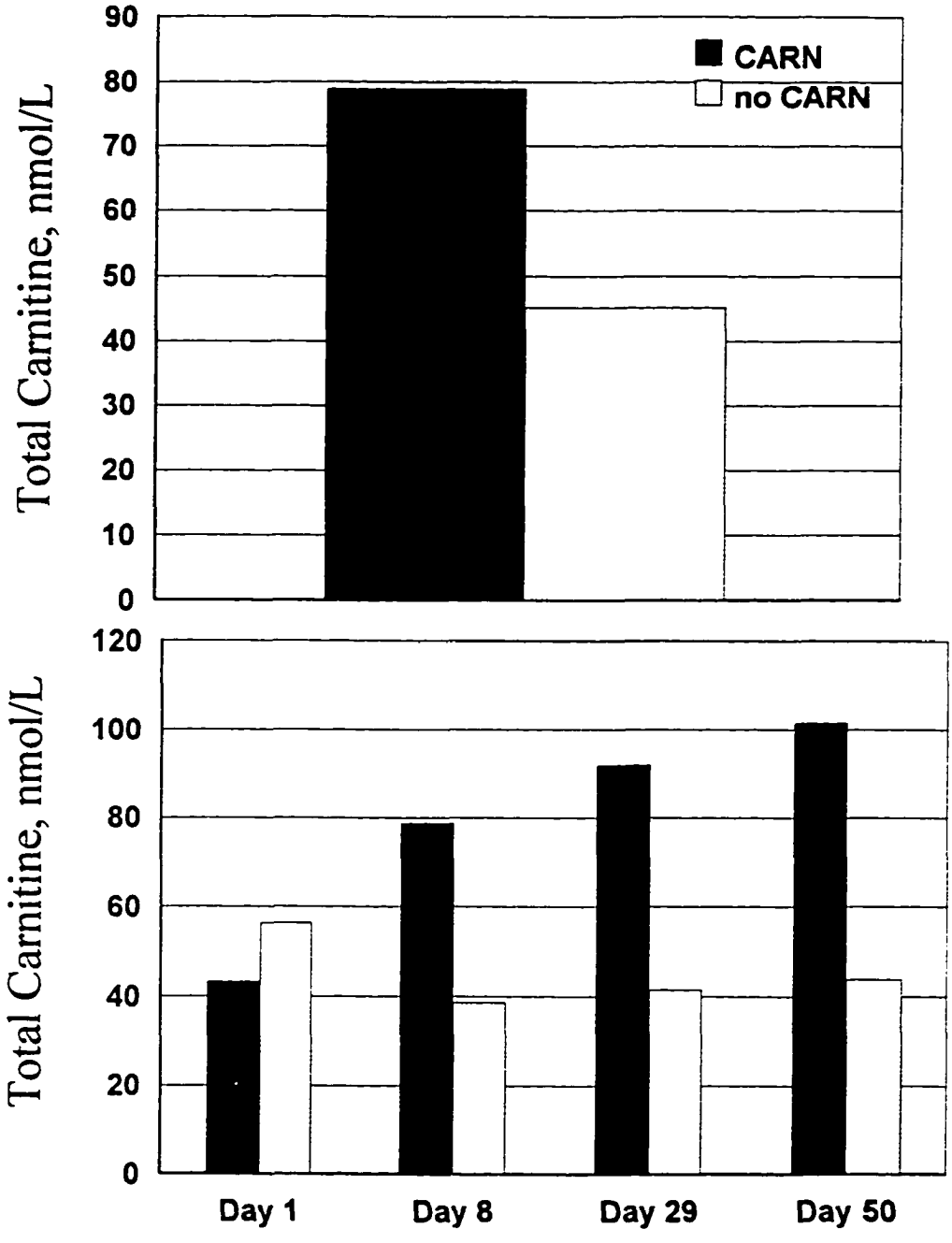

Figure 5.3. Effect of A) L-carnitine on plasma total carnitine and B) L-carnitine and day on plasma total carnitine. Experimental diets Carn = 499 ppm of L-Carnitine; no Carn = 0 ppm of L-Carnitine. The pooled SEM was 6.5 and 7.0 nmol/mL, respectively.

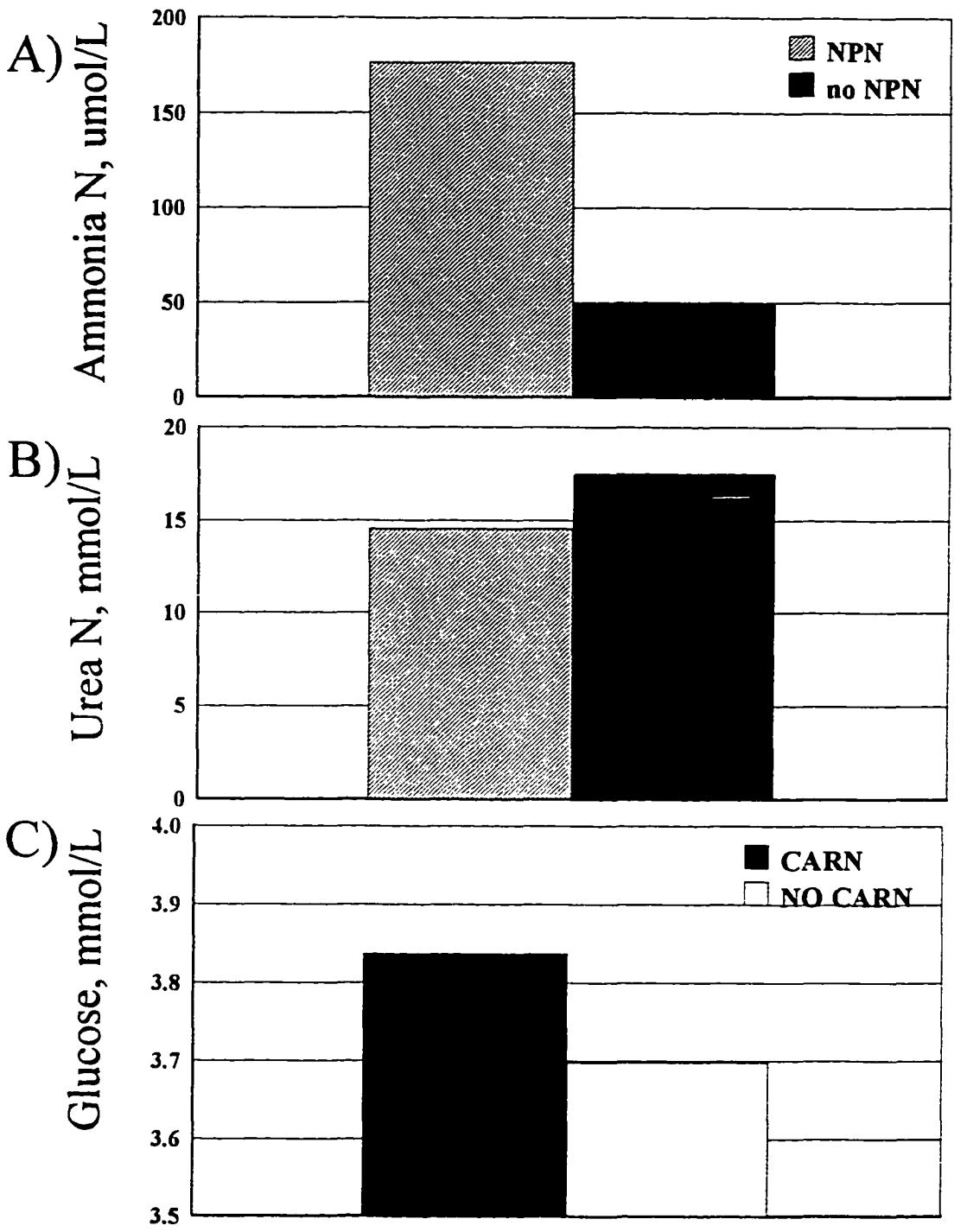

Figure 5.4. Effect of NPN and L-carnitine on plasma A) ammonia N, B) urea N, and C) glucose. Experimental diets were NPN = urea contributing 50% of the total dietary N; no NPN = urea contributing 0% of the total dietary N; Carn = 499 ppm of L-Carnitine; no Carn = 0 ppm of L-Carnitine. The pooled SE for ammonia N, urea N, and glucose were 9.1 μmol/L, 0.5 mmol/L, and 0.05 mmol/L, respectively.

Table 5.3. Effect of NPN and L-carnitine on plasma ammonia N, glucose, urea N, NEFA, and insulin concentrations in relation to feeding.

		Diets[a]		
Metabolite	Hr	no NPN	NPN	SEM
Ammonia N, μmol/L	0	59.3^{f}	74.6^{f}	8.9
	1	49.8^{xf}	259.0^{yg}	9.5
	3	39.2^{f}	194.9^{h}	8.2
Urea N, mmol/L	0	18.81^{xf}	11.65^{yf}	0.3
	1	18.32^{xf}	13.94^{yg}	0.3
	3	15.39^{xg}	17.82^{yh}	0.3
Glucose, mmol/L	0	3.79^{f}	3.79^{f}	0.03
	1	3.74^{f}	3.64^{g}	0.04
	3	3.99^{xg}	3.70^{yfg}	0.03
NEFA, μEq/L	0	143.4^{f}	115.4^{f}	7.5
	1	102.5^{xg}	149.6^{yg}	8.1
	3	82.0^{gh}	111.5^{f}	7.0
Insulin, μU/mL	0	35.3^{f}	43.1^{f}	8.0
	1	86.2^{xg}	46.4^{yf}	8.0
	3	103.4^{xgh}	59.6^{yf}	7.8

[a] Experimental diets were no NPN = urea contributing 0% of the total dietary N; NPN = urea contributing 50% of the total dietary N.

^{fgh} Within a column, means lacking a common superscript letter differ, $P < .01$.

^{xy} Within a row, means lacking a common superscript letter differ, $P < .0001$.

by Day 29 (NPN x Carn x Day, $P < .05$). Plasma albumin was not affected by Carn or NPN ($P > .10$). Lambs on the NPN diets showed 19% lower ($P < .02$) plasma T_4 concentrations (Figure 5.5A). Moreover, plasma T_4 levels increased from d 1 to 50 in ewe lambs, but decreased in the wether lambs from Day 1 to 29 increasing again at Day 50 (Sex x Day, $P < .04$). Plasma T_3 was not affected ($P > .10$) by either NPN or Carn (Figure 5.5B). However, the T_4:T_3 ratio was 15% lower ($P < .02$) in lambs fed diets containing NPN (Figure 5.5C). Ewe lambs fed the NPN diet had higher plasma cortisol compared with the diets without NPN; however, wethers fed NPN diets had lower cortisol compared with wethers fed diets without NPN (NPN x Sex; $P < 0.01$). Plasma insulin was higher in the lambs fed diets containing NPN at 1 and 3 hr post-feeding compared with lambs fed the NPN diets (NPN x Hr, $P < .002$; Table 5.3). There was a Carn x Day interaction ($P < .06$) for plasma insulin. Lambs on the Carn and NPN + Carn diets had the highest insulin concentrations on Day 1 and 8 compared with the lambs on the Cont and NPN diets, which had the highest insulin values on Day 1 and 29 (Figure 5.6).

Oral Urea Load Test. Samples drawn at the 0 min sample of the OULT 1 showed an effect of NPN. Ruminal fluid ammonia nitrogen ($P < .005$) and plasma urea nitrogen ($P < .0008$; Table 5.4) were lower in the lambs on the NPN diets. Plasma ammonia nitrogen ($P < .05$; Table 5.4) was higher in the lambs on the NPN diets. Lambs on the Carn diets had higher plasma glucose concentrations ($P < .04$; Table 5.4) compared with lambs on the non-Carn diets. At the 0 min sample, ruminal fluid pH was not affected by NPN ($P > .10$; Table 5.4). During the OULT 1, plasma ammonia nitrogen was higher in lambs on the NPN diets ($P < .0001$; Figure 5.7A). Ewe lambs had higher plasma

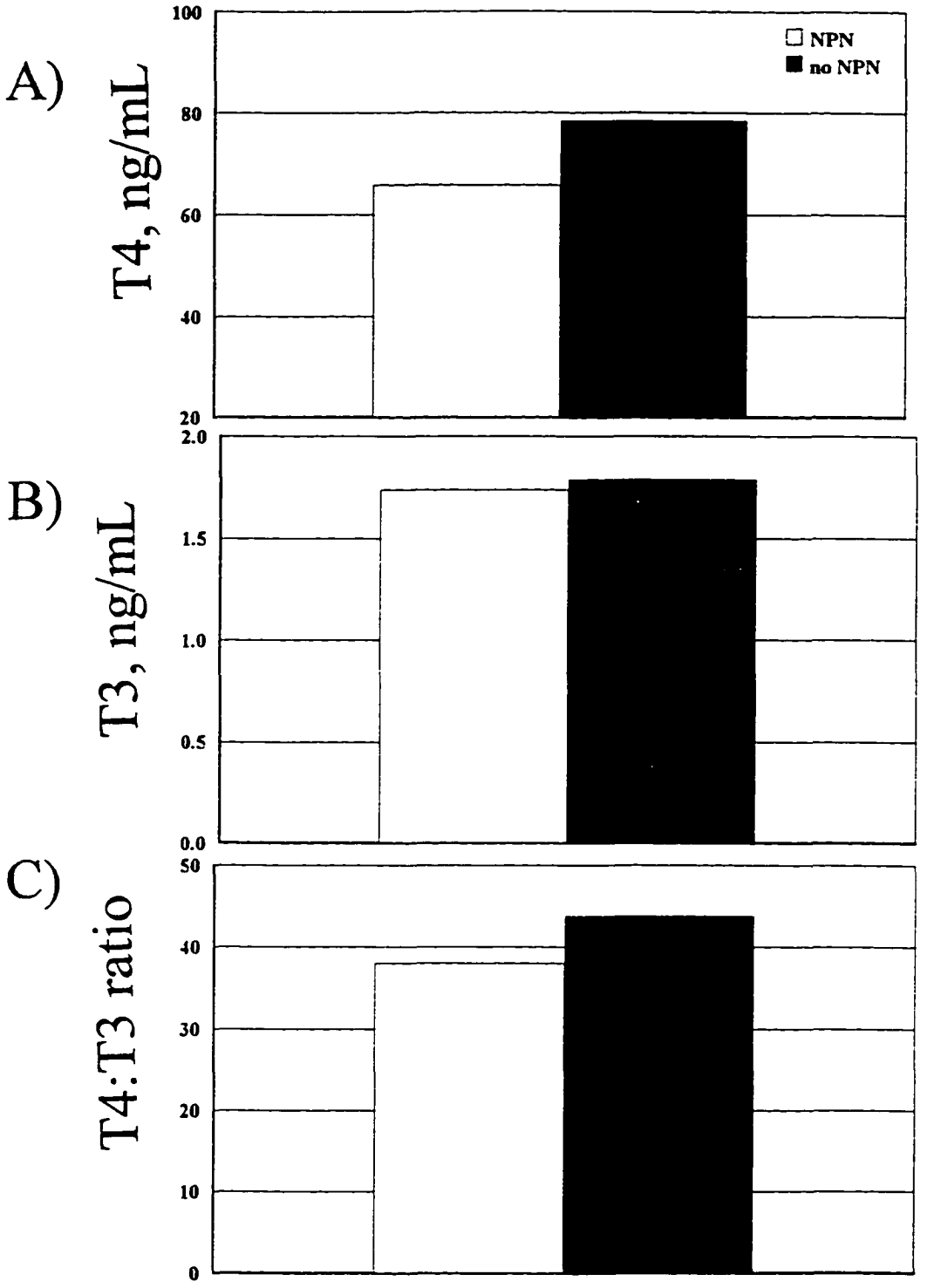

Figure 5.5. Effect of NPN and L-carnitine on plasma A) thyroxine (T_4), B) triiodothyronine (T_3), and C) T_4:T_3 ratio. Experimental diets were NPN = urea contributing 50% of the total dietary N; no NPN = urea contributing 0% of the total dietary N; Carn = 499 ppm of L-Carnitine; no Carn = 0 ppm of L-Carnitine. The pooled SE for T_4, T_3, and T_4:T_3 ratio were 1.3 ng/mL, 0.02 ng/mL, and 0.7, respectively.

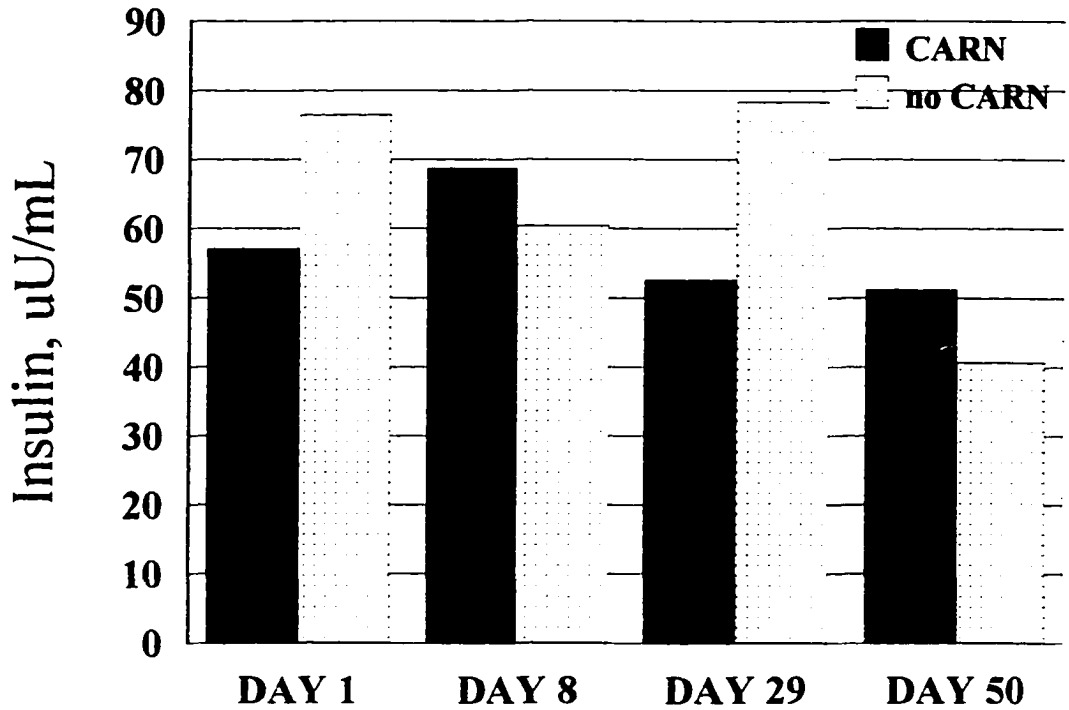

Figure 5.6. Effect of L-carnitine and day on plasma insulin concentrations. Experimental diets were Carn = 499 ppm of L-Carnitine; no Carn = 0 ppm of L-Carnitine. The pooled SE was 10.1 μU/mL.

Table 5.4. Effect of NPN and L-carnitine on concentrations of plasma and ruminal metabolites in samples collected prior (0 min sample) to an oral urea load test.

	Diets[a]				
Item	Control	NPN	Carn	NPN+Carn	SEM
OULT 1					
Ruminal Ammonia N, mmol/L[b]	5.85	11.27	5.68	10.98	2.1
Ruminal pH	6.6	6.7	6.6	6.7	0.04
Ammonia N, μmol/L[c]	64.0	68.4	59.4	75.4	2.4
Urea N, mmol/L[d]	21.90	14.07	20.43	14.69	0.8
Glucose, mmol/L[e]	3.52	3.52	3.81	3.86	0.07
Insulin, μU/mL	30.5	21.9	30.2	21.6	4.3
OULT 2					
Ruminal Ammonia N, mmol/L[b]	6.61	11.63	5.78	10.05	2.5
Ruminal pH	6.4	6.4	6.2	6.3	0.05
Ammonia N, μmol/L[c]	76.4	108.2	71.3	116.0	3.8
Urea N, mmol/L[d]	22.84	12.63	20.57	13.51	0.8
Glucose, mmol/L[e]	3.48	3.79	3.93	3.36	0.07
Insulin, μU/mL[f]	63.3	36.5	90.9	48.3	5.7

[a] Experimental diets were Control = urea contributing 0% of the total dietary N + 0 ppm L-Carnitine; NPN = urea contributing 50% of the total dietary N + 0 ppm of L-Carnitine; Carn = urea contributing 0% of the total dietary N + 499 ppm of L-Carnitine; NPN + Carn = urea contributing 50% of the total dietary N + 499 ppm of L-Carnitine.
[b] NPN effect, $P < .005$.
[c] NPN effect, $P < .05$.
[d] NPN effect, $P < .001$.
[e] Carn effect, $P < .05$.
[f] NPN effect, $P < .009$.

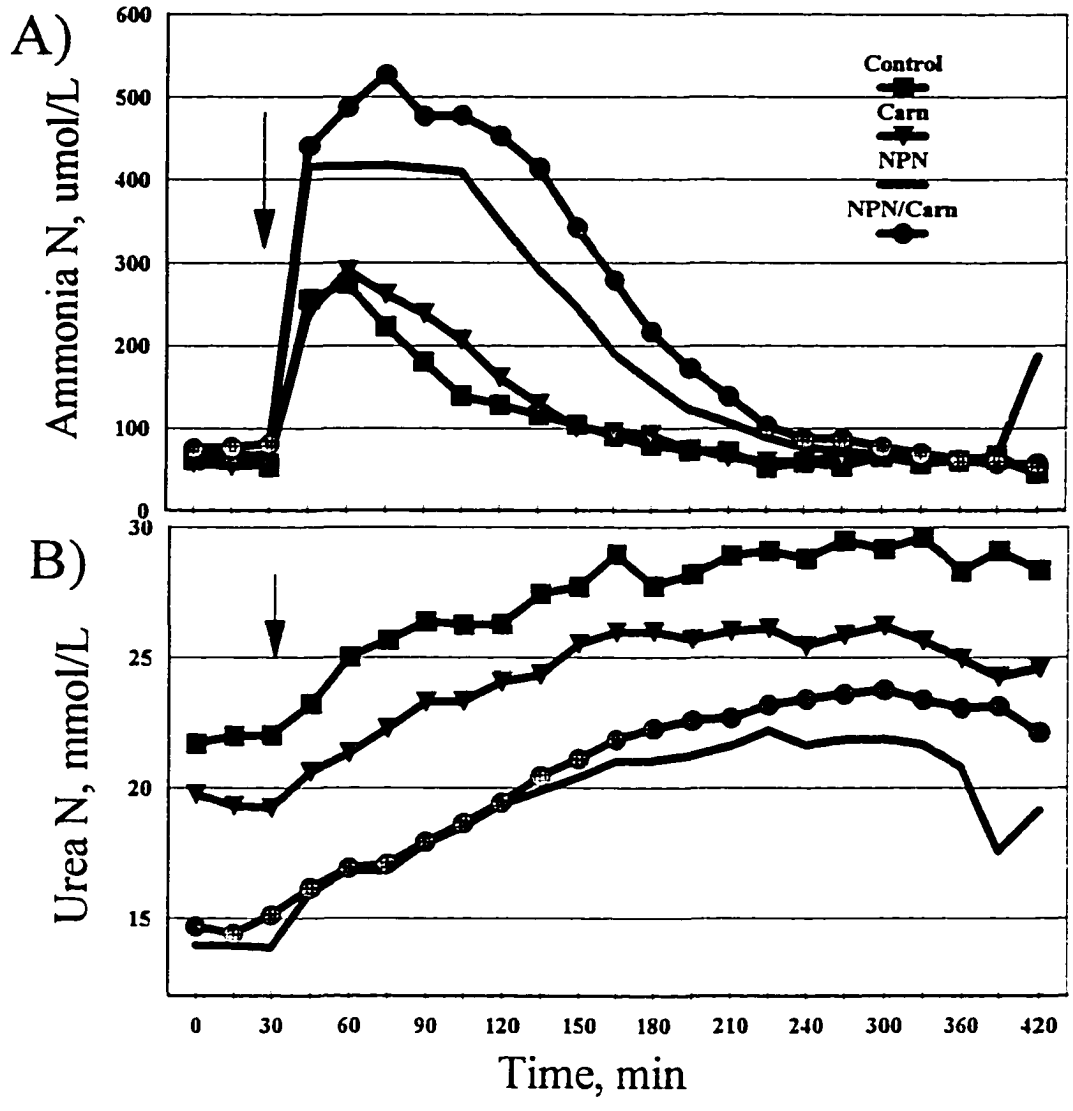

Figure 5.7. Changes in plasma A) ammonia N and B) urea N concentrations in sheep following an oral urea load test (OULT 1). Arrows denote when treatment was administered. Urea solution 0.835 g/kg[75] was administered via a stomach tube. The pooled SE for plasma ammonia N and urea N were 7.6 μmol/L and 0.3 mmol/L, respectively. Experimental diets were Control = urea contributing 0% of the total dietary N + 0 ppm L-Carnitine; NPN = urea contributing 50% of the total dietary N + 0 ppm of L-Carnitine; Carn = urea contributing 0% of the total dietary N + 499 ppm of L-Carnitine; NPN/Carn = urea contributing 50% of the total dietary N + 499 ppm of L-Carnitine.

ammonia nitrogen levels in the Cont, Carn, and NPN + Carn diets compared with wethers. However, ewe lambs consuming the NPN treatment had lower plasma ammonia nitrogen compared with the wethers (Carn x NPN x Sex, $P < .04$). Plasma urea nitrogen was lower in the lambs consuming the NPN diets ($P < .0001$; Figure 5.7B). Plasma glucose concentrations were higher in the Carn diets ($P < .0001$; Figure 5.8A) and in wethers ($P < .0001$). Plasma insulin was lowest in lambs consuming NPN diets compared with lambs not consuming NPN ($P < .0001$; Figure 5.8B).

Samples drawn at 0 min of the OULT 2 showed an effect of NPN. Ruminal fluid ammonia nitrogen ($P < .0001$), plasma urea nitrogen ($P < .0001$), and plasma insulin ($P < .009$) were lower in lambs consuming the NPN diets (Table 5.4). Plasma ammonia nitrogen ($P < .0002$) was highest in lambs consuming the NPN diets (Table 5.4). At the 0 min sample, ruminal fluid pH was not affected by NPN ($P > .10$; Table 5.4). During the OULT 2, plasma ammonia nitrogen was lower in the Carn and NPN + Carn diets when compared to the NPN diet ($P < .02$; Figure 5.9). Plasma urea nitrogen was lowest in lambs on the diets containing NPN ($P < .0001$; Figure 5.10A) compared to the lambs receiving diets containing supplemental NPN. Plasma glucose was lowest in the lambs fed the NPN diets ($P < .05$; Figure 5.10B); additionally, lambs fed both Carn and NPN had the lowest plasma glucose values (Carn x NPN, $P < .0003$). Plasma insulin concentrations were highest ($P < .007$) in lambs fed Carn and were lowest ($P < .0007$) in lambs fed NPN (Figure 5.10C). Plasma glucose was highest in the lambs fed the Carn diets but lowest in lambs fed the NPN + Carn diet (Carn x NPN, $P < .01$).

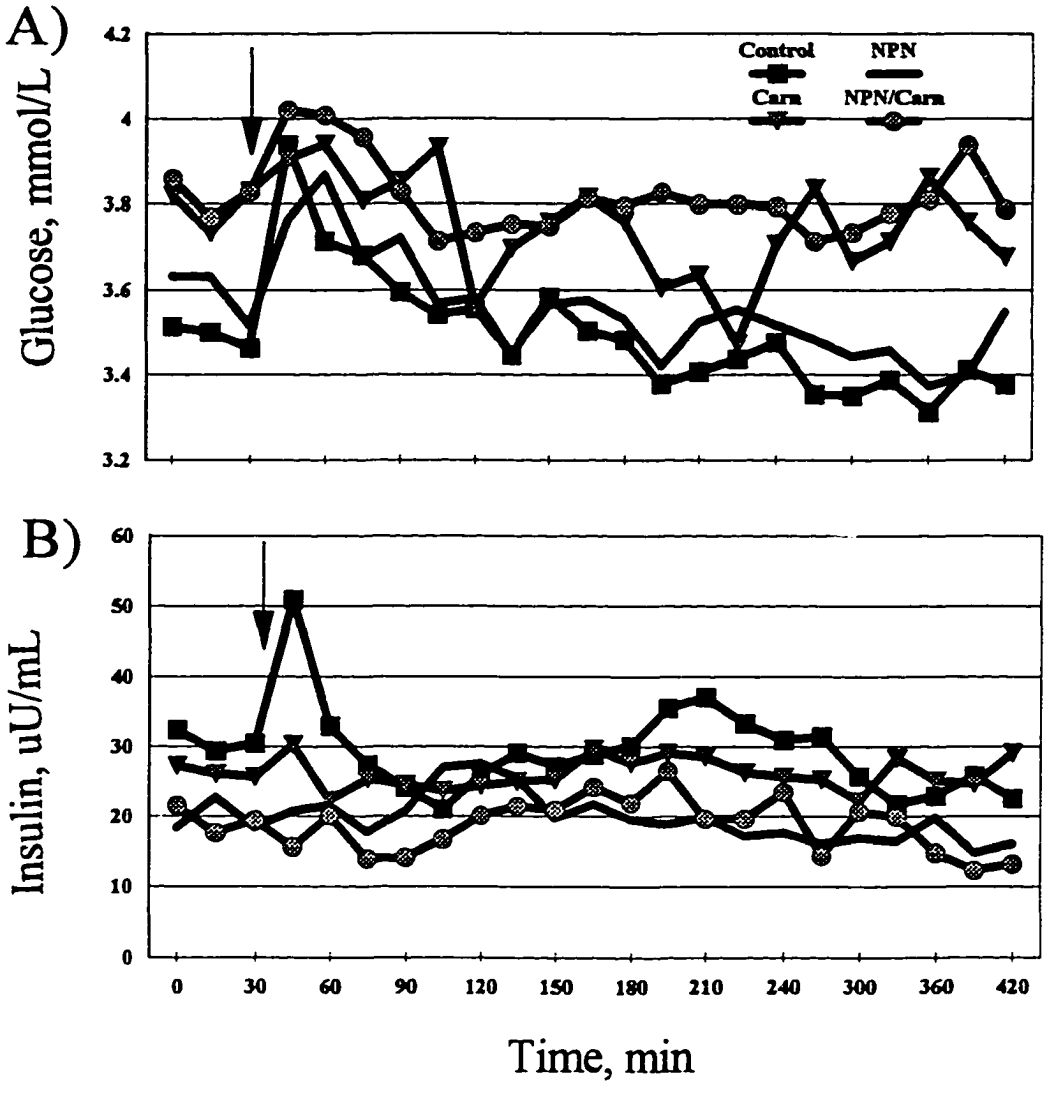

Figure 5.8. Changes in plasma A) glucose and B) insulin concentrations in sheep following an oral urea load test (OULT 1). Arrows denote when treatment was administered. Urea solution 0.835 $g/kg^{.75}$ was administered via a stomach tube. The pooled SE for plasma glucose and insulin were 0.03 mmol/L and 1.7μU/mL, respectively. Experimental diets were Control = urea contributing 0% of the total dietary N + 0 ppm L-Carnitine; NPN = urea contributing 50% of the total dietary N + 0 ppm of L-Carnitine; Carn = urea contributing 0% of the total dietary N + 499 ppm of L-Carnitine; NPN/Carn = urea contributing 50% of the total dietary N + 499 ppm of L-Carnitine.

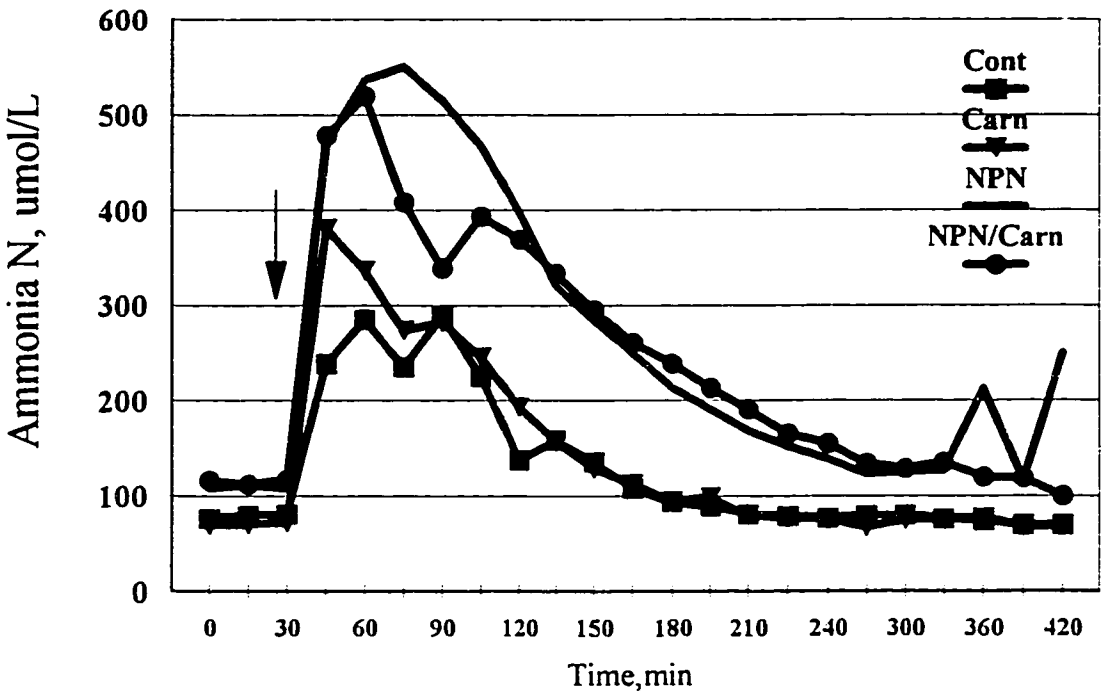

Figure 5.9. Changes in plasma ammonia N in sheep following an oral urea load test (OULT 2). Arrows denote when treatment was administered. Urea solution 0.835 g/kg[75] was administered via a stomach tube. The pooled SE for plasma ammonia N was 7.7 μmol/L. Experimental diets were Control = urea contributing 0% of the total dietary N + 0 ppm L-Carnitine; NPN = urea contributing 50% of the total dietary N + 0 ppm of L-Carnitine; Carn = urea contributing 0% of the total dietary N + 499 ppm of L-Carnitine; NPN/Carn = urea contributing 50% of the total dietary N + 499 ppm of L-Carnitine.

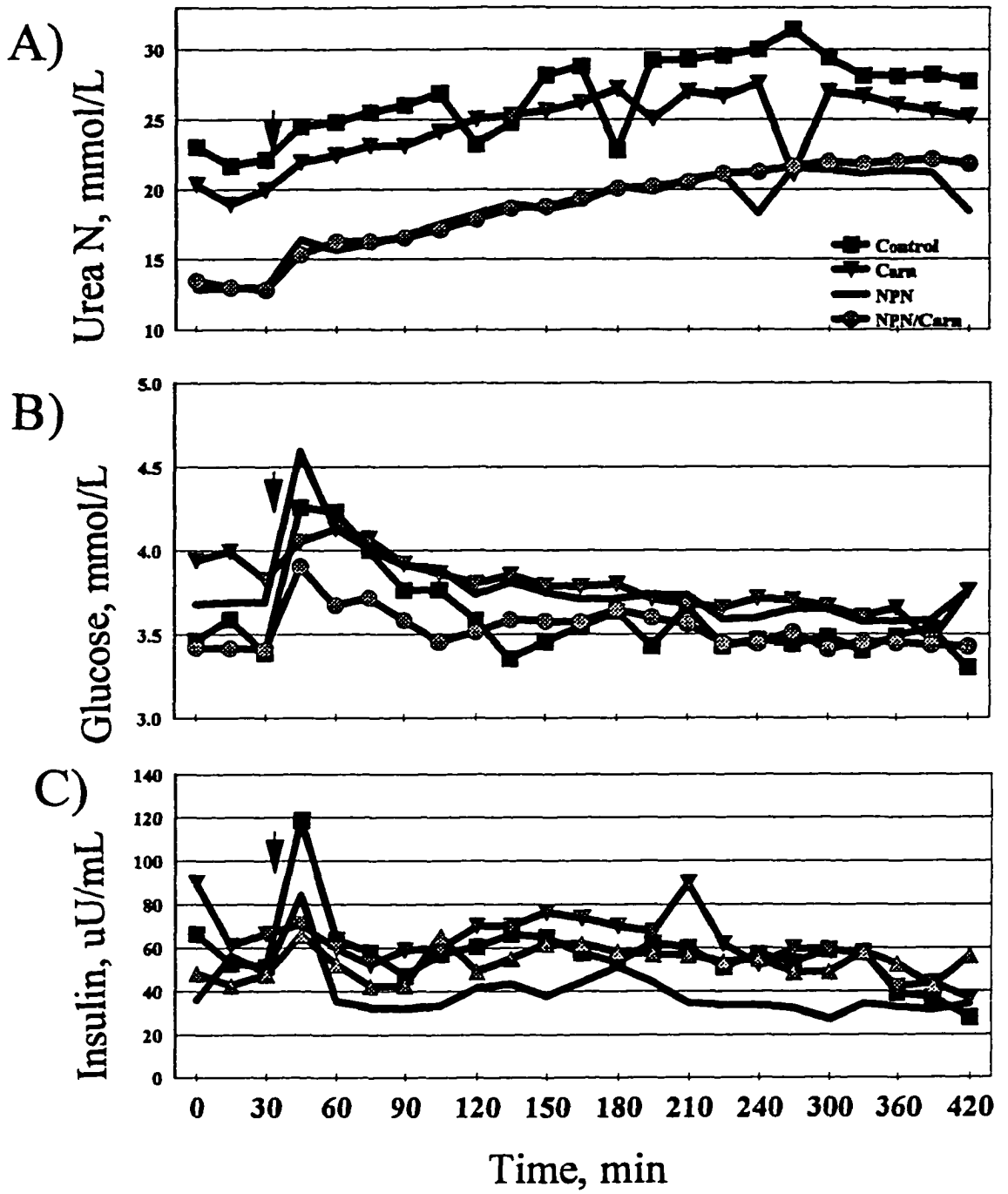

Figure 5.10. Changes in plasma A) urea N, B) glucose, and C) insulin concentrations in sheep following an oral urea load test (OULT 2). Arrows denote when treatment was administered. Urea solution 0.835 g/kg$^{.75}$ was administered via a stomach tube. The pooled SE for plasma urea N, glucose, and insulin were 0.4 mmol/L, 0.05 mmol/L, and 2.8 μU/mL, respectively. Experimental diets were Control = urea contributing 0% of the total dietary N + 0 ppm L-Carnitine; NPN = urea contributing 50% of the total dietary N + 0 ppm of L-Carnitine; Carn = urea contributing 0% of the total dietary N + 499 ppm of L-Carnitine; NPN/Carn = urea contributing 50% of the total dietary N + 499 ppm of L-Carnitine.

Discussion

Results of research conducted in pigs has shown that supplemental L-carnitine may be beneficial for production (Owen et al., 1996; Musser et al., 1997). However, the production and metabolic responses to supplemental L-carnitine have been variable in the ruminant. In this study, total feed intake, ADG, and gain:feed ratio were not affected by the addition of L-carnitine. However, in this study, an accurate measure of feed intake was difficut to achieve, due to the amount of feed that was spilled and unrecoverable for weigh-back. This agrees with a study conducted by Hill et al., (1994) who found no effect of supplemental L-carnitine on ADG, DMI, and gain:feed ratio in feedlot steers. These findings are in contrast to those of White et al. (1998) who found an improvement in ADG when L-carnitine was supplemented to grazing weanling calves; and DeRouen et al. (1998) who found that weaned beef calves fed broiler litter-corn diets plus L-carnitine had higher DMI and ADG. However, other studies reported decreases in ADG, DMI, and gain:feed ratio in weanling calves supplemented with L-carnitine and various protein sources (White et al., 1998) and Holstein calves fed broiler litter and L-carnitine (Yavuz et al., 1997). Although total feed intake was not different between treatments, feed intakes tended to be higher in lambs on Carn + NPN diet from Day 1 to 7. A similar trend was noted in weanling pigs. Owen et al. (1994b) found no overall effect on growth performance but found that pigs on 1,000 ppm L-carnitine were more efficient and heavier on d 35 postweaning compared with pigs not fed L-carnitine.

When NPN comprises a large percentage of the total dietary nitrogen in ruminant diets, cattle and sheep have exhibited reductions in growth, ADG, nitrogen retention, feed efficiency and milk production (Chalupa et al., 1970; Chalupa, 1972; NRC, 1976; Kertz et

al., 1982). Researchers have proposed that the decreased performance associated with the feeding of NPN may be due to derangements in intermediary metabolism (Prior et al., 1970; Chalupa, 1972; Spires and Clark, 1979; Emmanuel et al., 1982; Fernandez et al., 1988, 1997). In the present study, two of the treatments contained NPN which comprised 50% of the total dietary N. The sheep on the NPN diets showed a decrease in ADG and feed:gain ratio. In addition to affecting growth parameters, NPN affected the rumen characteristics to a greater extent compared to L-carnitine. Lambs which consumed the NPN containing diets had higher ruminal fluid pH. Feeding NPN as urea has been shown to increase rumen pH due to the rapid production of ammonia, through urea hydrolysis, which contributes to the alkalinity of the rumen fluid (Webb et al., 1972; Kertz et al., 1982). Ruminants fed diets containing NPN not only experienced increases in rumen pH, but also increases in ruminal ammonia nitrogen concentrations (Chalupa, 1972; Webb et al., 1972; Kertz et al., 1982; Fernandez et al., 1997). In the present study, lambs fed the NPN diets had higher ruminal ammonia nitrogen levels compared with lambs fed the non-NPN diets.

The combination of an increased rumen pH and ruminal ammonia nitrogen concentration would be reflected in the concentration of plasma ammonia nitrogen (Chalupa, 1972; Webb et al., 1972; Kertz et al., 1982). With an increase in rumen pH, excess ruminal ammonia nitrogen is absorbed across the rumen epithelium into the portal and lymphatic circulation (Edjtehadi et al., 1978; Spires and Clark, 1979; Symonds et al., 1981; Visek, 1984; Fernandez et al., 1990a). Plasma total carnitine values increased in the lambs fed the Carn diets compared with the lambs that were not fed Carn. This suggests that the L-carnitine is being absorbed by the lambs. Because the concentrations of total

carnitine increased throughout the collection period, this suggests that the rumen microorganisms showed minimal adaption to the supplemented L-carnitine. This is in contrast to LaCount et al. (1996a) who showed that carnitine degredation was greater in ruminal fluid from cows that had adapted to dietary carnitine supplementation for 2 weeks. Plasma ammonia nitrogen concentrations were highest in this study in lambs fed the NPN treatments. This suggests that the higher rumen pH and ruminal ammonia nitrogen affected the plasma ammonia nitrogen levels in these sheep. Because the plasma ammonia nitrogen concentrations in the NPN lambs averaged 177 μM, we considered these lambs to be experiencing subclinical hyperammonemia (Fernandez et al., 1988). Lambs fed the non-NPN diets did not show an increase in plasma ammonia nitrogen 1 hr post-feeding; this could be due to the soybean meal that was used as the protein source. Urea, which was the NPN source used in the present study, is rapidly hydrolyzed in the rumen by the rumen microorganisms (NRC, 1976). This rapid hydrolysis could explain why a rapid increase in plasma ammonia nitrogen was seen in lambs fed the NPN diets (NRC, 1976).

Supplementation of L-carnitine did not affect ruminal ammonia nitrogen or plasma ammonia nitrogen concentrations in this study. This agrees with Morris et al. (1998) who found that oral administration (1 g/d) of an L-carnitine solution in mature ewes for 10 days did not affect ruminal fluid ammonia nitrogen or plasma ammonia nitrogen concentrations. The ruminal and plasma ammonia nitrogen response to supplemental L-carnitine has been variable. Yavuz et al. (1997) showed that Holstein calves fed broiler litter and L-carnitine had higher ruminal ammonia nitrogen compared with calves fed a diet containing only broiler litter. In contrast, White et al. (1997) showed a decrease in ruminal ammonia nitrogen in grazing calves fed L-carnitine as a supplement. Plasma

ammonia nitrogen levels were not affected by L-carnitine in several trials conducted in calves (Yavuz et al., 1997; DeRouen et al., 1998; White et al., 1998).

Because ammonia is converted to urea in the liver, the expected response to high plasma ammonia levels is high plasma urea levels (Singh and Mudgal, 1984; Ropstad et al., 1989). Plasma urea nitrogen levels in the lambs fed NPN were lower in this study while L-carnitine had no effect on plasma urea nitrogen. One explanation may be that lambs in this study were fed at a level to meet the NRC protein requirements for growing lambs, so diets were balanced to meet these requirements. Therefore, lambs were not fed excess nitrogen which may explain why the high plasma ammonia nitrogen levels were not reflected in the plasma urea nitrogen concentrations (Preston et al., 1965; Gaskins et al., 1991). Another possibility is that the urea is being excreted. Sheep and cattle fed high levels of urea excrete more urea in the urine which lowers nitrogen retention (Chalupa, 1972). Some studies have shown decreases in plasma urea nitrogen levels with L-carnitine supplementation (Yavuz et al., 1997; White et al., 1997, 1998a,b) while other studies show no effect of L-carnitine on plasma urea nitrogen (DeRouen et al., 1998; Morris et al., 1998).

Supplemental L-carnitine affected plasma glucose concentrations in this study. Lambs fed L-carnitine had higher plasma glucose values. However, the response of plasma glucose to L-carnitine has also been variable. In a study conducted by Chapa et al. (1998), intravenous administration of L-carnitine (6.36 and 12.72 mmol/ kg $^{.75}$ BW) resulted in an increase in plasma glucose. Other studies have shown that plasma glucose either decreased (White et al., 1997,1998a), increased (Erfle et al., 1971; White et al., 1998a), or was not affected (LaCount et al., 1995; Yavuz et al., 1997; DeRouen et al.,

1998; Morris et al., 1998) by L-carnitine. In this study, there was no effect of NPN on plasma glucose, which suggests that the lambs on the NPN treatments were experiencing a slight hyperammonemia because one of the characteristics of hyperammonemia is hyperglycemia (Bartley et al., 1976; Emmanuel et al., 1982; Fernandez et al., 1988, 1990a,b). Plasma insulin was higher in the lambs fed the non-NPN diets at 1 and 3 hr post feeding. This does suggest that the lambs fed NPN were experiencing a subclinical ammonia toxicity since hypoinsulinemia has been associated with hyperammonemia (Barej and Harmeyer, 1979; Emmanuel et al., 1982; Fernandez et al., 1988). Insulin is a hormone that is known to inhibit lipolysis (Murray et al., 1990; Amaral-Phillips et al., 1993). It is not surprising that lambs fed NPN had increased plasma NEFA levels 1 hr post-feeding. Since plasma insulin levels were decreased, it is then possible for their plasma NEFA values to be elevated (Murray et al., 1990; Amaral-Phillips et al., 1993). Nevertheless, insulin was not affected in lactating does fed diets containing up to 50% of the total N in the form of urea (Fernandez et al., 1997).

The thyroid hormones, T_3 and T_4, are associated with regulation of metabolic rate and modulation of growth- and metabolism-related hormones (Brockman and Laarveld, 1986). Plasma T_4 was lower in lambs fed NPN, and therefore, the T_4:T_3 ratio was lower due to the NPN diets. This suggests that the metabolic rate was increased in the NPN lambs since T_3 has greater biological activity compared to T_4 (Murray et al., 1990). Sex affected plasma T_4 values with ewe lambs showing higher levels of this hormone compared with wethers. Sex and NPN affected plasma cortisol concentrations; ewe lambs fed NPN had higher plasma cortisol and wethers fed NPN had lower plasma cortisol. Fernandez et al. (1997) found no effect of NPN on cortisol concentrations in lactating goats.

Two OULT were conducted during this study. An early OULT was conducted at Day 10 of the collection period (OULT 1) and a late OULT was conducted at d 50 of the collection period (OULT 2). During the OULT 1, plasma ammonia nitrogen was highest in the lambs fed NPN. Morris et al. (1998) found that oral administration of L-carnitine prior to an OULT did not decrease plasma ammonia nitrogen. This is in contrast to Chapa et al. (1997) who found that intravenous L-carnitine administration prior to an OULT prevented an increase in plasma ammonia nitrogen concentrations in response to the bolus of urea. The effectiveness of L-carnitine may be influenced by the route of administration or the adaptation of the rumen microbial population to L-carnitine. O'Connor et al. (1986) found that, in mice, intraperitoneal injections of L-carnitine offered the most protection against ammonia toxicity followed by intravenous and intramuscular administration. LaCount et al. (1996a) found that carnitine degradation was greater in ruminal fluid from cows that had adapted to dietary carnitine supplementation for 2 weeks. In the present study, total carnitine concentrations almost doubled from baseline concentrations by Day 7 in the lambs fed Carn, however, this level of carnitine in the plasma may not have been effective in lowering the plasma ammonia nitrogen concentration. The results of the OULT 2, during which plasma ammonia nitrogen concentrations were lower in the lambs fed Carn and NPN+Carn could be attributed to the increase of plasma total carnitine. By Day 50 of the trial, at the time of the OULT 2, total carnitine nearly tripled from baseline concentrations. The low plasma insulin could be in response to the induced hyperammonemia which is associated with hypoinsulinemia (Barej and Harmeyer, 1979; Emmanuel et al., 1982; Fernandez et al., 1988). Lambs fed L-carnitine had higher plasma glucose concentrations during both OULT 1 and 2. These

results are in contrast to Chapa et al. (1997) and Morris et al. (1998) who found no effect of L-carnitine on plasma glucose levels during an OULT.

In summary, ADG and gain:feed ratio were lower in lambs fed the NPN diets. Incorporation of NPN in the diets increased ruminal fluid pH and ammonia nitrogen, increased plasma ammonia nitrogen and lowered plasma urea nitrogen and T_4. Addition of L-carnitine increased plasma total carnitine concentrations but had minimal effects on ruminal and other blood characteristics. Supplemental L-carnitine tended to increase feed intake early in the experimental period. Although L-carnitine did not affect plasma ammonia nitrogen concentrations early in the feeding trial (OULT 1), L-carnitine did lower plasma ammonia nitrogen later in the feeding trial (OULT 2).

Implications

Nonprotein nitrogen incorporation in the diets affected both production parameters and blood metabolites. Plasma total carnitine did increase with supplemental L-carnitine. However, addition of L-carnitine to the diets had minimal effects on ruminal and other blood characteristics. L-carnitine tended to improve feed intake early in the experimental period. Although plasma ammonia nitrogen levels were not affected by L-carnitine early in the feeding trial (OULT 1), L-carnitine did lower plasma ammonia nitrogen later in the feeding trial (OULT 2). This suggests that supplementation of L-carnitine with NPN may prevent hyperammonemia in ruminants. It is possible that the supplementation of L-carnitine may be more beneficial in ruminants consuming a poor quality diet compared to those on a feedlot type of diet. Since the lambs in this study were fed protein at a level to

meet their requirements for growth, it is possible that L-carnitine may also be beneficial in ruminants consuming excess protein. Further research is warranted into elucidating the ideal dose of L-carnitine and the most effective route of administration.

Chapter 6

Overall Summary and Conclusions

Livestock production and metabolism have been shown to be negatively affected by excess soluble nitrogen (Chalupa, 1972; Visek, 1984; Elrod and Butler, 1991). Reductions in feed intake, growth, ADG, milk production, and fertility are production parameters that are evidence of the negative effects of excess soluble nitrogen (Chalupa, 1972; NRC, 1976; Kertz et al., 1982; Elrod and Butler, 1991). Metabolically, excess soluble nitrogen may result in subclinical ammonia toxicity which causes derangements in intermediary metabolism (Chalupa, 1972; Spires and Clark, 1979; Visek, 1984).

The results from the first experiment suggest that the nutritional status of dairy cows prior to grazing seemed to have a more of an effect on the ability of the cow to maintain body condition during early lactation than the composition of protein supplement fed throughout early lactation and the grazing period. Body condition and nitrogen status seemed to be negatively affected by the protein supplement containing excess total protein; however, other metabolic parameters were minimally affected by any of the protein supplements. The second experiment showed that intravenous administration of L-carnitine significantly lowered plasma ammonia nitrogen levels in ewes given an oral urea challenge even though the concentration of rumen free, nonionized ammonia nitrogen were similar to the ewes treated only with the urea solution. This suggests that L-carnitine administration may prevent hyperammonemia in ruminants. However, the possible mechanism by which this protection is afforded remains unknown.

In the third experiment, which was conducted to determine the influence of supplemental L-carnitine on growth and metabolic criteria of growing lambs fed a diet high in NPN, ADG and Gain:feed ratio were lower in lambs fed the NPN. Incorporation of NPN in the diets increased ruminal pH and ammonia nitrogen, increased plasma ammonia nitrogen and lowered plasma urea nitrogen and thyroxine. Addition of L-carnitine increased the plasma total carnitine concentration and tended to improve feed intake early in the experimental period. However, L-carnitine had minimal effects on ruminal and other blood characteristics. Although plasma ammonia nitrogen concentrations were not affected by L-carnitine early in the feeding trial (OULT 1), L-carnitine did lower plasma ammonia nitrogen later in the trial (OULT 2). Results of the OULT 2 suggest that supplementation of L-carnitine with NPN may prove useful in the prevention of hyperammonemia in ruminants.

It is clear that soluble nitrogen exerted deleterious effects on production parameters and influenced selected blood metabolites in growing lambs and grazing dairy cows. By lowering the plasma ammonia nitrogen levels in OULT 2 and influencing glucose metabolism, L-carnitine may prove to be useful in preventing ammonia toxicity. These results suggest that further research is needed to elucidate the mechanisms by which L-carnitine may be affording protection against ammonia toxicity. Additionally, further study is needed to determine the best dosage and route of administration of L-carnitine.

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Vita

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DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Angelica Maris Chapa

Major Field: Animal Science

Title of Dissertation: Dietary Nitrogen and Energy Metabolism in the Ruminant: Effect of Source of Nitrogen

Approved:

J. Marcos Fernandez
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Dean of the Graduate School

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